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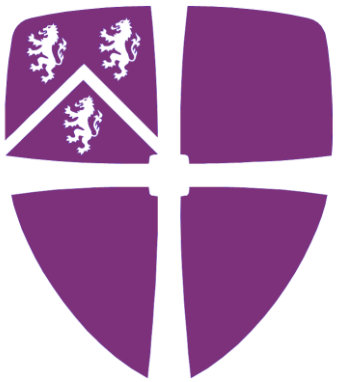

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Christopher Brown

Leishmaniasis is an insect vector-borne Neglected Tropical Disease (NTD) caused by the protozoan *Leishmania spp*. This disease is endemic in 98 countries, claiming 30,000 lives each year and putting a further 350 million people at risk. With no vaccine currently available, treatment typically requires long, expensive courses of exposure to toxic medicines *via* parenteral administration. Furthermore, resistance to some antileishmanials is an emerging threat. There is an urgent need for novel treatments that are inexpensive and free of side effects.

Our group has previously identified and validated the *Leishmania major* inositol phosphorylceramide synthase (*Lmj*IPCS) enzyme as an attractive drug target. This essential membrane-bound enzyme has a differential function to the mammalian orthologue (sphingomyelin synthase), potentiating the development of safe, selective antileishmanials. Using a microtiter plate compatible assay, a set of 1040 pharmacologically active compounds were screened for activity against *Lmj*IPCS and *L. major* parasites. Clemastine, an over-the-counter medicine, was found to be effective against *L. major* promastigotes (ED_{50} = 0.47 μM) and amastigotes (ED_{50} = 3.06 μM). With well-studied pharmacokinetic and mammalian safety profiles, clemastine was chosen as an ideal candidate for further development.

Initial efforts focussed on the synthesis of a series of chimeric analogues, coupling 2-pyrrolidinyl and 4-chlorobenzhydryl fragments *via* ether, triazole, amide, ester and olefin linkers. Amides presented the most attractive linker group, proving to be robust and amenable to parallel synthetic efforts. Despite being well-tolerated by the *Lmj*IPCS enzyme, the synthesised amides exhibited low *in cellulo* activity against *L. major* promastigotes.

Two series of amide-linked clemastine analogues were synthesised, exploring substitution of the pyrrolidine and benzhydryl moieties. From a synthesised library of 59 compounds, the indazole *N*-(1''-[2'''-dimethylaminoethyl]indazol-6''-yl)-2-(4'-chlorophenyl)-2-phenylacetamide exhibited *Lmj*IPCS inhibition (IC_{50} = 2.41 μM) comparable to the parent molecule, clemastine (IC_{50} = 2.87 μM). However, this analogue proved to be ineffective against *L. major* promastigotes. In an attempt to restore antiparasitic action, the amide was replaced by a flexible ether linker, providing 1-(2'-[dimethylamino]ethyl)-6-([{4''-chlorophenyl}{phenyl}methoxy]methyl)indazole. This analogue exhibited inhibition of *Lmj*IPCS activity, with an IC_{50} of 4.72 μM, but only hindered growth of *L. major* promastigotes above 5 μM.

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Abbreviations

4 Å MS:	4 Å molecular sieves
AbA:	aureobasidin A
Al:	alkyl
Amp B:	amphotericin B
aq.:	aqueous
Ar:	aryl
AU:	arbitrary units
bipy:	2,2'-bipyridine
Boc:	*tert*-butoxycarbonyl
Cbz:	benzyloxycarbonyl
CDC-DPDx:	Center for Disease Control and Prevention, Division of Parasitic Diseases
CHAPS:	3-[(3-cholamidopropyl)dimethyl ammonio]-1-propanesulfonate
CI:	confidence interval
CL:	cutaneous leishmaniasis
conc.:	concentration
COSY:	^{1}H-^{1}H correlation spectroscopy
DAG:	diacylglycerol
DALY:	disability-adjusted life year
DCE:	1,2-dichloroethane
DCM:	dichloromethane
DMAP:	4-dimethylaminopyridine
DMEDA:	*N*,*N*'-dimethylethylenediamine
DMF:	*N*,*N*-dimethylformamide
DMSO:	dimethylsulfoxide
DND*i*:	Drugs for Neglected Diseases *initiative*
ED_{50}:	median effective dose
EDCI:	N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide
eq.:	equivalent(s)
GCMS:	Gas chromatography-mass spectrometry
h:	hour(s)
HDAC:	histone deacetylase
HMBC:	^{1}H-^{13}C heteronuclear multiple bond correlation
HOBt:	1-hydroxybenzotriazole
HPTLC:	high performance thin-layer chromatography
HTS:	high-throughput screening
IC_{50}:	half maximal inhibitory concentration
IM:	intramuscular
IPC:	inositolphosphoryl ceramide
IPCS:	inositolphosphoryl ceramide synthase
IR:	infrared
IV:	intravenous
KDAC:	lysine deacetylase
LCMS:	Liquid chromatography-mass spectrometry
LD_{50}:	median lethal dose
La:	*Leishmania amazonensis*
Ld:	*Leishmania donovani*
lit.:	literature value
Lmj:	*Leishmania major*
Lmx:	*Leishmania mexicana*
LPP:	lipid phosphate phosphatase
MCL:	mucocutaneous leishmaniasis
min:	minute(s)
MOA:	mode of action
M.p.:	melting point
MRCT:	Medical Research Council Technology Ltd.

MS:	mass spectrometry
Ms:	methanesulfonyl
NBD:	nitrobenzoxadiazole
NCE:	new chemical entity
NGO:	non-governmental organisation
NINDS:	National Institute of Neurological Disorders and Stroke
NMM:	*N*-methylmorpholine
NMR:	nuclear magnetic resonance
NMT:	*N*-myristoyl transferase
NTD:	neglected tropical disease
PATH:	Program for Appropriate Technology in Health
PCC:	pyridinium chlorochromate
PDP:	Product Development Partnership
PI:	phosphatidylinositol
PO:	per os
ppm:	parts per million
p-TSA:	*para*-toluenesulfonic acid
RT:	room temperature
s:	second(s)
SAR:	structure-activity relationship
SL:	sphingolipid
SLS:	sphingolipid synthase
SM:	sphingomyelin
SMS:	sphingomyelin synthase
SPT:	serine palmitoyl transferase
TEMPO:	2,2,6,6-tetramethylpiperidine-1-oxyl
TFA:	trifluoroacetic acid
THF:	tetrahydrofuran
TLC:	thin-layer chromatography
TMS:	trimethylsilyl
TR:	trypanothione reductase
Ts:	*para*-toluenesulfonyl
UV:	ultraviolet
VL:	visceral leishmaniasis
VT:	variable temperature
WHO:	World Health Organisation
WT:	wild type

Declaration

The work described in this thesis was carried out in the Department of Chemistry, Durham University; Medical Research Council Technology Ltd., Mill Hill, London; and the London School of Hygiene and Tropical Medicine, London between October 2011 and September 2015. All work is the author's own, except for collaborative research, which is acknowledged as appropriate. This work has not been previously submitted for a degree at this or any other institution.

Statement of Copyright

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Finally, my deepest gratitude goes to my partner Sarah and my family, for the unending support and advice they have given me.

1. Introduction

1.1 Overview

The work within this thesis is aimed towards the discovery and development of potential chemotherapeutic agents for the treatment of the neglected parasitic disease leishmaniasis (caused by *Leishmania spp.*). The specific target of this project is the protozoan inositol phosphorylceramide synthase (IPCS), specifically *Leishmania major* IPCS. This enzyme presents an attractive drug target by virtue of the lack of a direct mammalian homologue, offering the possibility of drug candidates with low host toxicity.

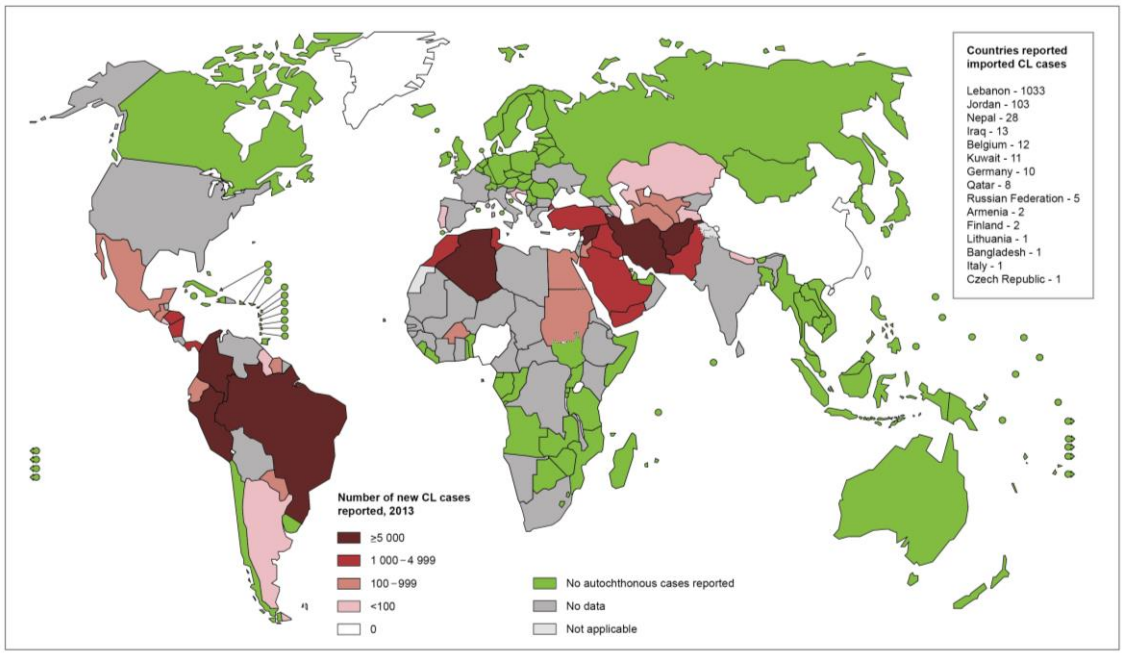
This thesis comprises 9 chapters. Chapters 2 – 7 will discuss the work undertaken and the chemical and biological experimental procedures developed in these studies will be detailed in Chapters 8 and 9, respectively. The remainder of this first chapter outlines the background to the project.

1.2 The Leishmaniases

Neglected Tropical Diseases (NTDs) are a group of 17 diseases which are prevalent in poorer populations of the world, infecting over 1 billion people.[1,2] A large proportion of these diseases result from parasitic infection. These diseases have been classified as 'neglected' due to their predominance in areas of poverty, as well as their relatively low priority on national and international health agendas.[2]

Infection by obligate kinetoplastid parasites of the genus *Leishmania* cause a range of clinical manifestations collectively termed the leishmaniases. The disease is endemic in over 98 countries and 3 territories, putting 310 million people at risk, with an estimated 1.3 million new cases emerging annually (Figure 1–1).[3,4]

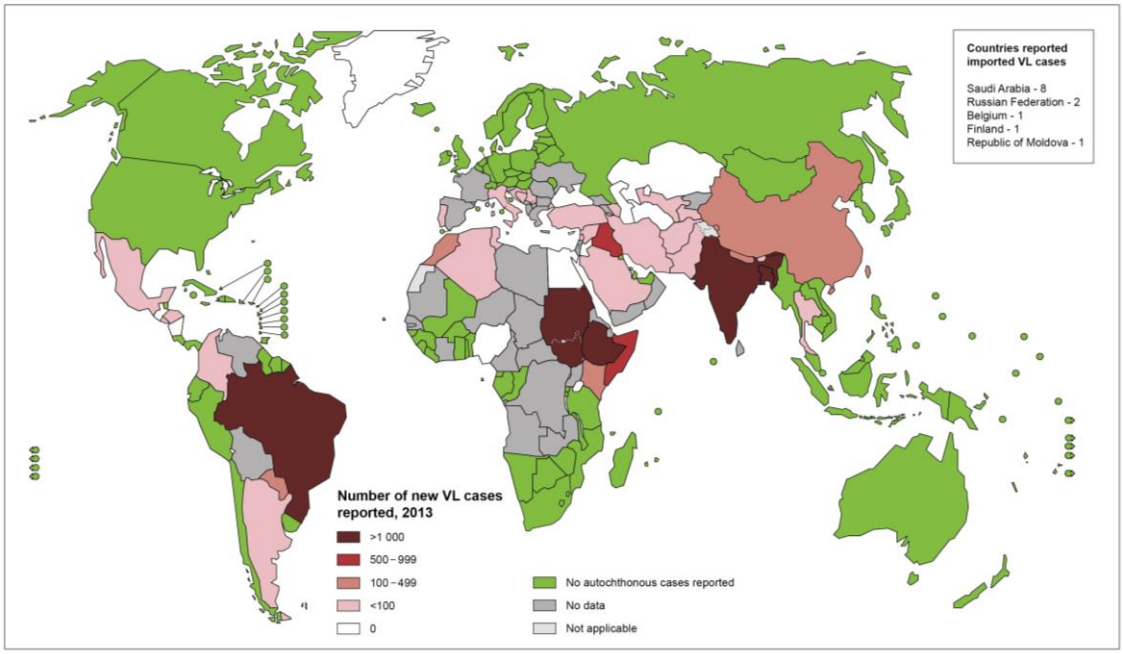
Status of endemicity of cutaneous leishmaniasis, worldwide, 2013



Data Source: World Health Organization
Map Production: Control of Neglected Tropical Diseases (NTD)
World Health Organization

World Health Organization

Status of endemicity of visceral leishmaniasis, worldwide, 2013



Data Source: World Health Organization
Map Production: Control of Neglected Tropical Diseases (NTD)
World Health Organization

World Health Organization

Figure 1–1: Endemicity of cutaneous leishmaniasis and visceral leishmaniasis in 2013. Reproduced, with permission of the publisher, from the World Health Organisation (WHO) Global Health Observatory map gallery (http://www.who.int/gho/map_gallery/en/)

A conservative estimate of 30,000 deaths occur each year due to *Leishmania* infection.[3] A more accurate indication of the impact of leishmaniasis is given by disability-adjusted life years (DALYs), as they take into account the morbidity of disease. Collectively, the leishmaniases inflict a loss of 3.32 million DALYs, accounting for 13% of all NTD-related DALYs lost.[5,6] This impact is further compounded by the economic burden on already poverty-stricken areas, through restricted child development and crippled adult worker productivity.[5,7]

1.2.1 Life cycle of *Leishmania spp*.

The human infective variants of leishmaniasis are caused by about 21 of 30 species that infect mammals. These include the *L. donovani* complex with 3 species (*L. donovani*, *L. infantum*, and *L. chagasi*); the *L. mexicana* complex with 3 main species (*L. mexicana*, *L. amazonensis*, and *L. venezuelensis*); *L. tropica*; *L. major*; *L. aethiopica*; and the subgenus *Viannia* with 4 main species (*L. (V.) braziliensis*, *L. (V.) guyanensis*, *L. (V.) panamensis*, and *L. (V.) peruviana*).[8]

These parasites reside in the midgut of the *Phlebotomus* and *Lutzomyia* sandfly vectors (Figure 1–2). In their log-phase procyclic promastigote form, they proliferate until they reach stationary phase. The parasites then transform into infective metacyclic promastigotes (a process called metacyclogenesis), which are transmitted to humans as the sandfly takes a blood meal. In the bloodstream they are phagocytised by macrophages, where they transform into their non-motile amastigote form, residing within the acidic phagolysosome. These amastigotes multiply and infect other tissues, giving rise to the clinical manifestations of *Leishmania* infection. The cycle is propagated when a sandfly ingests amastigote-infected blood.

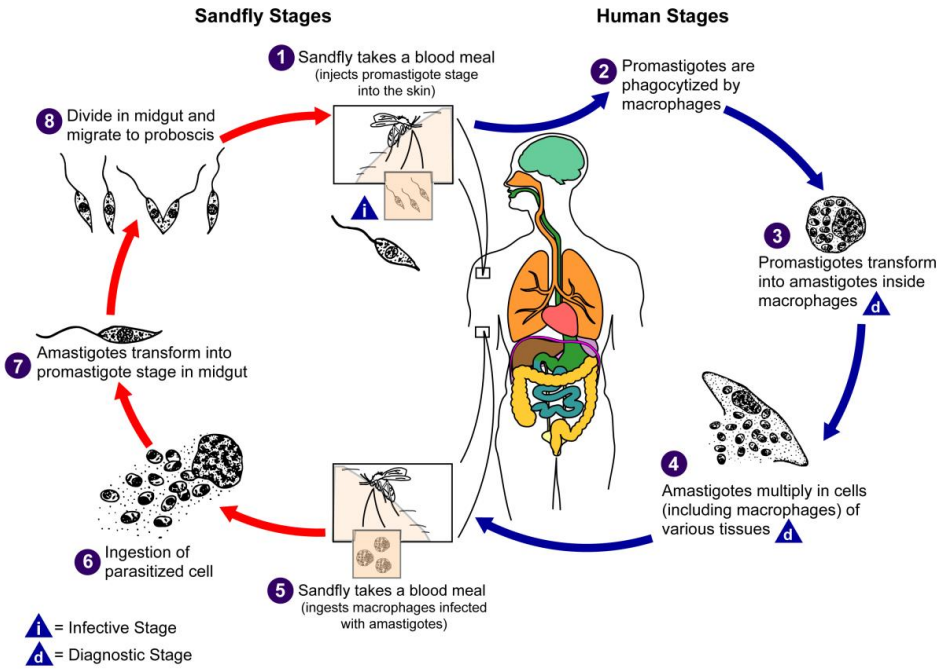


Figure 1–2: Life cycle of *Leishmania spp.* Image provided by the Center for Disease Control and Prevention - Division of Parasitic Diseases (CDC-DPDx).

1.2.2 Symptoms of leishmaniasis

Three main disease states are possible, depending on the infective species. Cutaneous leishmaniasis (CL), most commonly caused by *L. major*, presents as ulcerations that may heal spontaneously. In more extreme cases, as with recidivans and diffuse cutaneous leishmaniasis, these lesions are chronic and difficult to treat.[2]

Mucocutaneous leishmaniasis (MCL), caused mostly by *Leishmania* species of the *Viannia* subgenus, is the most disfiguring form involving the destruction of soft tissue in the nose, mouth and throat. Whilst they are non-fatal, CL and MCL can be extremely debilitating and stigmatise those afflicted.[2]

By contrast, visceral leishmaniasis (VL), caused mainly by *L. donovani*, manifests as fever, hepatosplenomegaly and pancytopenia. It is the most severe form of Leishmaniasis; if left untreated it is always fatal. Even if effective treatment is given, the patient may then suffer from a cutaneous form known as post-kala azar dermal leishmaniasis.[2]

1.2.3 Current therapies

There is currently no approved vaccine against *Leishmania* infection,[5] so control of the disease relies on a limited number of drug treatments (Figure 1–3).

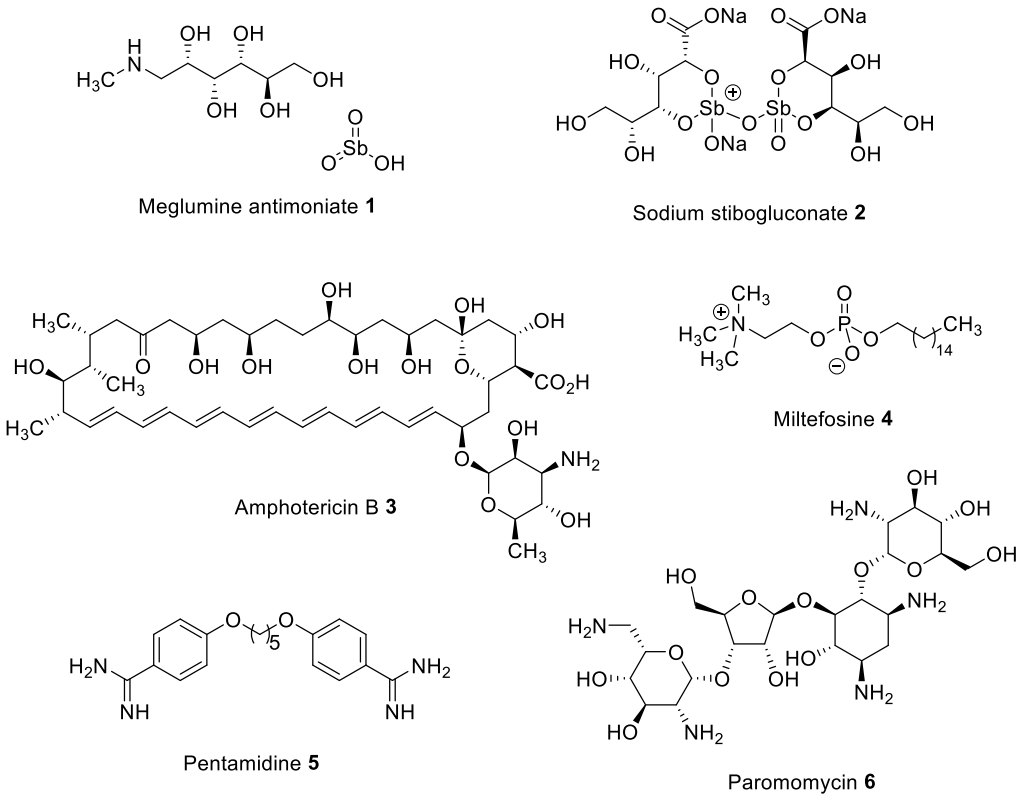


Figure 1–3: Current therapies for leishmaniasis

Key information on the individual therapies is provided in Table 1–1. Although a detailed description of the merits and challenges of current antileishmanial treatment is beyond the scope of this thesis, there exist a number of comprehensive reviews for further reading.[9–11]

Generally, the available drugs for leishmaniasis suffer from a number of inadequacies. The majority of treatments require slow, often painful injections of active ingredients which carry significant toxicity issues. Although efforts have been made to mitigate specific issues, such as the advent of liposomal formulations of amphotericin B and the development of miltefosine (the only orally available antileishmanial), it remains that no single therapy for leishmaniasis exists that does not have a significant drawback.

Table 1–1: Current therapies for leishmaniasis

Drug	Route	Mode of action (MOA)	Toxicity	Advantages	Disadvantages
Sb^{V} **1**, **2**	IV, IM	Sb^{III}: trypanothione reductase;[12] Zn-finger proteins;[13] increasing DNA fragmentation;[14] increasing intracellular Ca^{II} concentration.[15] Sb^{V}: stimulating macrophage microbicidal mechanisms.[16]	Cardiotoxicity; pancreatitis; hepatotoxicity; nephrotoxicity.[17]	Readily available; low cost (~$60).[9]	Long, painful treatment; toxicity; resistance in India.[18]
Amphotericin B **3**	IV	Complexes ergosterol to disrupt membranes.[19]	Severe nephrotoxicity.[20]	High efficacy (>97%); low cost (~$20)	Slow injection; high toxicity; thermal instability; resistance appearing in clinical isolates.[21]
Amphotericin B **3** (liposomal)	IV		Low nephrotoxicity	Well tolerated; reduced toxicity	Very high cost ($280–3000); slow injection; thermal instability
Miltefosine **4**	PO	Fatty acid and sterol metabolism;[22] cytochrome-*c* oxidase inhibition;[23] immunomodulatory inhibition of PI3k/Akt.[24]	Mild gastrointestinal effects; teratogenicity; nephrotoxicity.[25]	Only oral drug available	Susceptibility differs across *Leishmania* species;[26] long half-life (resistance risk);[27] teratogenic; relatively high cost ($65–150).[9]
Pentamidine **5**	IM	Polyamine biosynthesis and uptake,[28] DNA binding.[29]	Death; diabetes mellitus; cardiotoxicity; nephrotoxicity.[30]		High toxicity; low efficacy;[11] resistance.[31]
Paromomycin **6**	IM (VL), topical (CL)	Inhibition of polyphenylalanine synthesis.[32]	Mild hepatotoxicity; ototoxicity.[33]	Low cost ($15).[9]	Painful injections;[33] resistance in laboratory isolates;[34] variation in efficacy.[35,36]

It is clear that there is an urgent need for antileishmanial treatments that are efficacious, orally available, cheap and do not suffer from significant toxicity or a high potential for resistance. The lack of such drugs results in part from underinvestment in drug discovery by the pharmaceutical industry. Between 2000 and 2011, only 3.8% of all registered drugs listed neglected or tropical diseases as the principal indication.[37]

The past two decades have seen an increase in efforts toward controlling the global burden of neglected diseases. Product Development Partnerships (PDPs) such as Drugs for Neglected Diseases *initiative* (DND*i*), PATH and the Sabin Vaccine Institute PDP were established between 1999 and 2011. These partnerships aim to co-ordinate the efforts of academia, the pharmaceutical industry, the public sector and philanthropic organisations towards drug development.[38] Furthermore, in 2012, leading pharmaceutical companies, charities and NGOs announced their commitment to achieve goals set out by the WHO to control or eliminate ten NTDs by 2020.[39]

1.2.4 Drugs in clinical trials

Until novel therapies for leishmaniasis are brought to market, combination therapies have been investigated as a means of increasing efficacy and tolerance, as well as reducing treatment duration, cost and resistance potential.[17,40] Combination therapies based on liposomal amphotericin B **3**, miltefosine **4**, paromomycin **6** and pentavalent antimonials **1** and **2** are being developed and implemented throughout Africa, Asia and South America.[41–43]

Assessing different formulations of available drugs has been used as another method of addressing their drawbacks. The liposomal formulation of amphotericin B **3** successfully curtailed serious toxicity concerns and other reformulations have been investigated.[44,45] Clinical trials revealed a reduction in creatinine levels (indicative of lower nephrotoxicity), chills, rigors and nausea, when compared to conventional amphotericin B desoxycholate.[46] Recently, a pilot study in Iran focussed on the application of liposomal formulations of meglumine antimoniate **1** and paromomycin **6**.[47]

Some new therapies are currently going through clinical trials, either as monotherapies or in combination with existing antileishmanials (Table 1–2 and Figure 1–4).

Table 1–2: Prospective new therapies for leishmaniasis in clinical trials

Drug	Description	MOA	Trials
Sitamaquine **7**	Oral VL treatment	No defined mode of action.[48]	Phase II trials have been carried out in Africa.[49] A recent phase II study in India displayed cure rates comparable to amphotericin B **3**.[50]
Fexinidazole **8**	Oral VL treatment	Putative activation by nitroreductases.[51]	Recently terminated phase II trial in Sudan due to lack of efficacy.[52] Combination therapy with miltefosine **4** currently being evaluated.[53]
Pentoxifylline **9**	Oral CL treatment	Possible host immunomodulation.[54]	Combination treatment with Sb^V **1** and **2** against MCL encouraging,[55] but lacking against CL.[56] Further Phase II/III trials taking place,[57] including combination therapy with miltefosine **4**.[58]
Gentamicin **10** (WR 279396)	Topical combination therapy with Paromomycin **6**	Not leishmanicidal, but postulated to aid host immunity.[59]	A series of Phase II trials studying New and Old World CL have been completed.[60,61] Phase III trials are in progress.[62,63]
Imiquimod **11**	Topical combination therapy	Boosts macrophage production of cytokines and NO.[64]	Combination therapy with Sb^V **1** and **2** not definitive.[65,66] A study with oral miltefosine were conducted in Bolivia, but no results have been published.[67]
Allopurinol **12**	Oral treatment	Putative inhibitor of protein synthesis;[68] Purine salvage inhibitor.[69]	Not effective as a monotherapy.[70] Combination treatment with Sb^V **1** and **2** against CL has potential,[71] and trials continue.[72]
Azithromycin **13**	Oral treatment	Unknown in *Leishmania*.[73]	Clinical trials against CL infection proved ineffective.[74,75]
Fluconazole **14**	Oral treatment	Ergosterol biosynthesis inhibitor.[76]	Successful phase II trials of monotherapeutic fluconazole **14** have been carried out against CL,[77,78] highlighting minor side effects (cheilitis and nausea). Phase III trials are underway.[79]
Terbinafine **15**	Oral or topical treatment	Ergosterol biosynthesis inhibitor.[80]	Oral treatment and topical treatment in combination with Sb^V **1** and **2** promising.[81,82] Phase I trials underway in Iran.[83,84]

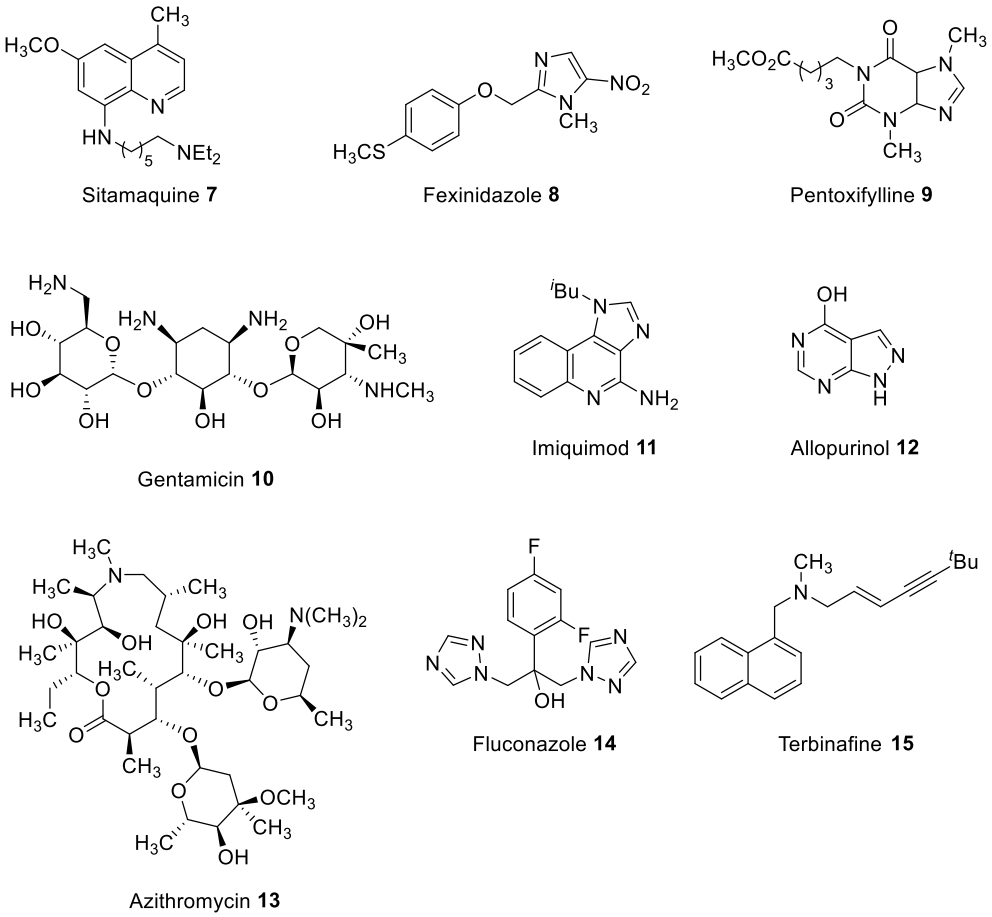


Figure 1–4: Prospective new therapies for leishmaniasis

Overall, the majority of current clinical trials are focussed on reformulating or combining existing antileishmanial treatments, or combination therapy with prospective new drugs. Of the new treatments being trialled, few have a clearly defined mode of action in *Leishmania*. As such, the potential for resistance through modification of protein targets, drug transport, and drug efflux remains a concern.[70] Access to a diverse arsenal of drugs with different mechanisms of action and no cross-resistance will help avoid this problem. Despite the efforts described above, there remains a dearth of studies into novel therapies for leishmaniasis, and no new chemical entities (NCEs) are being trialled.[37]

1.2.5 Drug discovery efforts

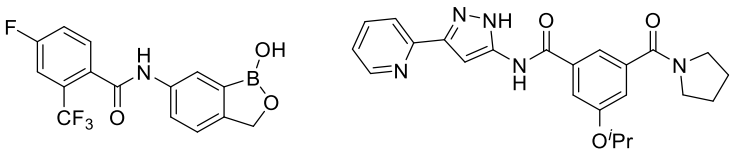
With the advent of PDPs and commitment of the pharmaceutical industry to tackling NTDs, there are a number of potential new therapies for leishmaniasis on the horizon. A detailed analysis of the NTD drug discovery process is provided by a number of recent reviews.[10,40,85–87]

The following section will summarise the most promising candidates, and the paradigms used to discover them.

1.2.5.1 Phenotypic screening

The resources available to medicinal chemists tackling neglected diseases are limited when compared to conventional drug discovery. As such, researchers have had to work around a lack of funding, tools, validated targets and suitable assays.[38] One way of doing this is through high-throughput screening (HTS) of existing compound libraries (owned by private or public sector organisations) in whole cell assays against target organisms in a phenotypic assay (the desired phenotype being reduced cell viability).[29]

Phenotypic screening campaigns have been used to discover NCEs with antiparasitic activity but unknown MOA (Figure 1–5). As part of a collaboration between Anacor Pharmaceuticals and DND*i*, oxaborole SCYX-6759/AN-4169 **16** was found to have micromolar activity against intracellular *L. donovani* amastigotes.[88] Discovered in their Human African Trypanosomiasis drug discovery program, the compound also displayed a good pharmacokinetic profile in rats and primates.[89] Further work identified a compound named DND*i*-2035804 (structure unavailable), which despite showing good clearance of *L. donovani* in an animal model, failed toxicity screening.[90] Similarly, aminopyrazoles, such as compound **17**, were discovered in a Pfizer compound library screen,[38] with reductions in parasitaemia of up to >99% being found in a hamster VL model. Studies continue with an aim to identify a lead compound in 2016.[91]

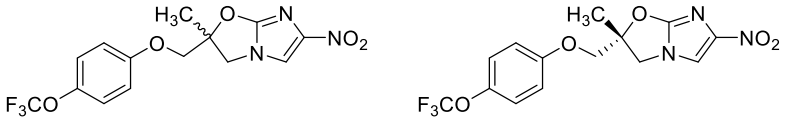


SCYX 6759/AN 4169 **16** Pfizer aminopyrazole **17**

Figure 1–5: Example hit compounds from phenotypic screening

Nitroaromatic compounds have gained much attention for their potential as antiparasitics.[92] Nitroimidazole DND*i*-VL-2001 **18** was identified from a library of anti-tubercular compounds as having potent leishmanicidal activity (ED_{50} = 0.03 µM), in a screen against luciferase-expressing *L. donovani* (Figure 1–6).[93] Specifically, the *R*-enantiomer, DND*i*-VL-2098 **(*R*)-18**, displayed efficacy against *L. braziliensis* and *L. major* at sub-micromolar levels. DND*i*-VL-2098 **(*R*)-18** exhibited high parasite clearance *in vivo*,[93] and a favourable pharmacokinetic profile, with high

permeability, good oral bioavailability (37% – 100%) and a half-life of up to 6 h.[94] However, recently discovered testicular toxicity in some animal models halted its progression to clinical trials.[95] DND*i* is currently investigating two nitroimidazooxazines DND*i*-0690 and DND*i*-8219 (structures unavailable) as successors for oral treatment.[96]



DND*i*-VL-2001 **18** DND*i*-VL-2098 **(*R*)-18**

Figure 1–6: Nitroimidazole antileishmanial compounds

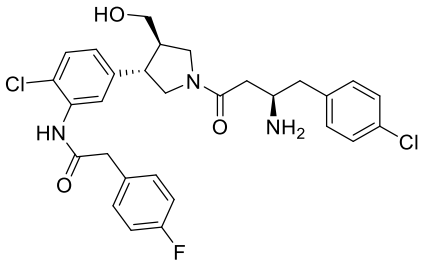
Further pharmacophores discovered through whole cell phenotypic screening include azoles,[97–99] chalcones,[100,101] quinazolines,[102,103] and quinolines.[104,105]

Phenotypic screening campaigns have been preferred for NTD research due to their pragmatic nature. Hits from these screens are guaranteed to have drug-like properties under physiological conditions, potentially expediting pharmacokinetic optimisation.[106] Furthermore, hits may act on several targets within the parasite, potentially reducing resistance risk. Nevertheless, there are some limitations to this strategy. Promastigote assays are still heavily relied upon for HTS campaigns due to their ease of operation. However, it has been estimated that only 4% of hits against promastigotes have an effect on intracellular amastigotes, highlighting their significant differences in biochemistry.[107] Reflecting this, suitable intramacrophage assays are increasing in number, in an attempt to reduce the risk of false results.[108–110] Advances in genomics and proteomics have improved target identification capability,[111–113] but this may not be straightforward, especially when more than one molecular interaction is involved.[114] This in turn may hamper optimisation of hit compounds.[115]

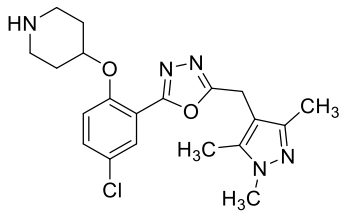
1.2.5.2 Target-based strategies

Despite the popularity of phenotypic screening approaches, target based strategies are important for the discovery of leishmanicidal compounds. Identifying protozoan enzymes which have no (or a substantially different) human ortholog can aid the development of selective drugs with low host toxicity.[116] Further understanding of targets can be gained, assisted by advances in the 'omics'.[112,113] For example, studies across species, and at different life-cycle stages, can help identify broad-spectrum antileishmanials.[116] Some of the approaches employed in the rational design of potential drugs are discussed below.

N-Myristoyl transferase (NMT) enzymes, which catalyse protein modification with myristic acid, have been discovered as an attractive target for antitrypanosomal agents.[117] Recently, Hutton *et al.* published work on the development of a potent and selective inhibitor of *L. donovani* NMT.[117] Hits from a high-throughput screen against *Ld*NMT were identified, before X-ray crystallography and *in silico* approaches guided the synthesis of a novel inhibitor **19** with K^i = 1.6 nM and a selectivity index (SI) of 17 against the human NMT1 (Figure 1–7). Unfortunately, cytotoxic activity against *L. donovani* extracellular amastigotes was not sufficient for further development (ED_{50} = 10 – 30 µM), this was attributed to poor cellular uptake. A separate study into *Plasmodium falciparum* NMT inhibitors revealed compound **20** as a potent *Ld*NMT inhibitor (K^i = 0.01 µM) (Figure 1–7).[118] Once again however, this compound did not exhibit the desired leishmanicidal activity, displaying an ED_{50} of >50 µM against axenic *L. donovani* amastigotes.



NMT inhibitor **19**



NMT inhibitor **20**

Figure 1–7: *Leishmania N*-myristoyl transferase inhibitors

The trypanothione redox system, unique to kinetoplastids, has been the centre of much activity. Research by Fairlamb *et al.* highlighted trypanothione reductase (TR) as an essential enzyme in *Leishmania*.[119,120] A number of compound classes which inhibit TR have been identified through *in vitro* and *in silico* work (Figure 1–8).[121,122] However, issues of low selectivity and poor translation to antileishmanial activity have hindered further progress.[121] The wide, relatively featureless active site of TR and its similarity to the human homologue, glutathione reductase, are possible reasons for the observed poor selectivity.[85,123] Other potential targets in the trypanothione pathway are the essential enzymes trypanothione synthetase and tryparedoxin peroxidase.[85]

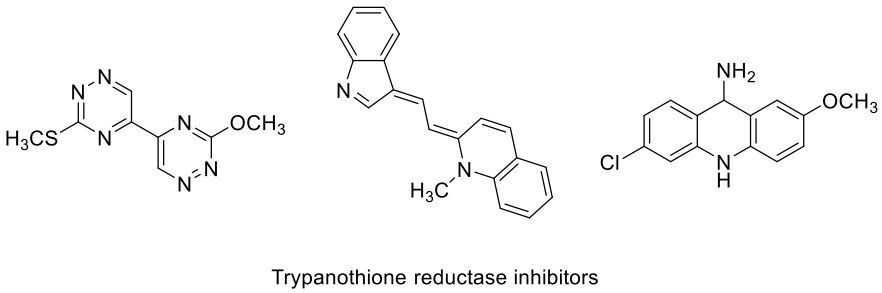


Figure 1–8: Inhibitors of *Leishmania* trypanothione reductase

Other putative antileishmanial drug targets have been reviewed recently;[10,85,116] notable examples include kinases,[124–126] sterol biosynthesis,[127,128] folate biosynthesis;[129,130] topoisomerases,[131–133] and proteases.[134,135]

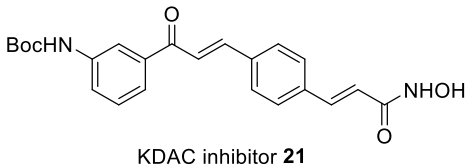
In spite of these efforts, it remains that target based approaches are generally less successful than phenotypic screening methods.[136] One key criticism is the disparity between potency against enzyme and target organism.[115] This may be due to instability within the acidic parasitophorous vacuole,[137] ineffective penetration of the parasite's unique cell surface,[138] or susceptibility to removal *via* metabolism or efflux.[115,137] With the comparative lack of resources available for NTD drug discovery, it remains a large endeavour to develop a rationally designed drug molecule with a specific mode of action.

1.2.5.3 'Piggy-back' approaches

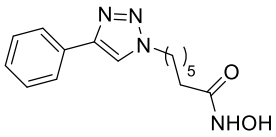
Another strategy, known as 'piggy back' drug discovery, makes use of phenotypic and target-based approaches to exploit compounds which have known biological function in other organisms.[87] Provided the target parasite has an orthologous enzyme, these compounds may provide a good starting point for hit to lead studies.

Histone deacetylase (HDAC) enzymes are popular targets in oncological drug discovery; two inhibitors are currently approved for T-cell lymphoma treatment in the US, and other potential anti-cancer drugs are in clinical studies.[139] A recent study was conducted in which known inhibitors of human lysine deacetylase (KDAC) enzymes were tested against a range of parasitic pathogens.[140] Hydroxamate **21** was found to have sub-micromolar activity against axenic *L. donovani* amastigotes (Figure 1–9). Future aims are centred on identifying *Leishmania* KDACs and *in silico* homology modelling with human enzymes to inform the development of selective inhibitors. In a separate investigation into HDAC inhibitor analogues, triazole **22** was found to have modest activity against *L. donovani* promastigotes

(EC_{50} = 15.8 µM) (Figure 1–9).[141] These studies highlight deacetylase enzymes as potential therapeutic targets for *Leishmania* infection.



KDAC inhibitor **21**



HDAC inhibitor **22**

Figure 1–9: Deacetylase enzyme inhibitors discovered *via* a 'piggy back' approach

Recently, a high-throughput screen of the GlaxoSmithKline diversity set of 1.8 million compounds was conducted against *L. donovani* at 5 µM.[142] The potential modes of action for hits were found by comparing known human protein targets with the parasite genome; from this, kinases were revealed as the most common target, alongside proteases, cytochromes and phosphodiesterases. Over the past few years, vast amounts of data from such phenotypic screens have been made publically accessible.[143] In addition, some compound sets are freely available for use, circumventing a previous road block to academic researchers.[144]

1.2.5.4 Drug repurposing, repositioning and rescue

Discovering and developing a NCE drug is estimated to cost ~$US800 million and take up to 20 years.[87] The investigation of already licenced medicines has become a popular method of shortening the drug discovery process for neglected diseases. A drug may be repurposed (applied to a new disease), repositioned (used as a template for further development against a new indication), or rescued (applied to a new disease after being abandoned from its original use). These terms are generally used interchangeably. The main advantage of this technique is that candidates have passed through several stages of clinical trials and hence have well understood pharmacokinetic and safety profiles.[145,146]

This is not a new concept in antileishmanial drug development, most of the currently used medicines were initially utilised against other diseases. Amphotericin B **3** is a repurposed antifungal treatment,[147] paromomycin **6** a broad spectrum antibiotic,[148] and miltefosine **4** a failed antineoplastic agent.[149] Furthermore, many of the therapeutics undergoing clinical trials (Table 1–2 above) are currently licenced for other indications. A recent screen of agrochemicals against *L. donovani* and the suggestion that veterinary medicines are an untapped resource highlight the fact that research is extending beyond repurposing human therapeutics.[87,150]

1.3 Inositol phosphorylceramide synthase (IPCS)

1.3.1 Sphingolipids

Sphingolipids (SLs) are a class of amphipathic lipids which form a major component of eukaryotic membranes, particularly the plasma membrane.[151] Additionally, they are key signalling components, implicit within a range of biological processes including cell growth, membrane trafficking and apoptosis.[152–154] SLs are characterised by an amino alcohol backbone unit, termed a sphingoid base, with varying head group functionalities and *N*-acyl substitution (Figure 1–10). Sphingoid bases differ vastly across nature, with variations in unsaturation, hydroxylation and alkyl chain tail being observed.[155] Because of this, there exists significant diversity in the structures of the principal sphingolipid species of mammals, fungi and plants, and the kinetoplastids.

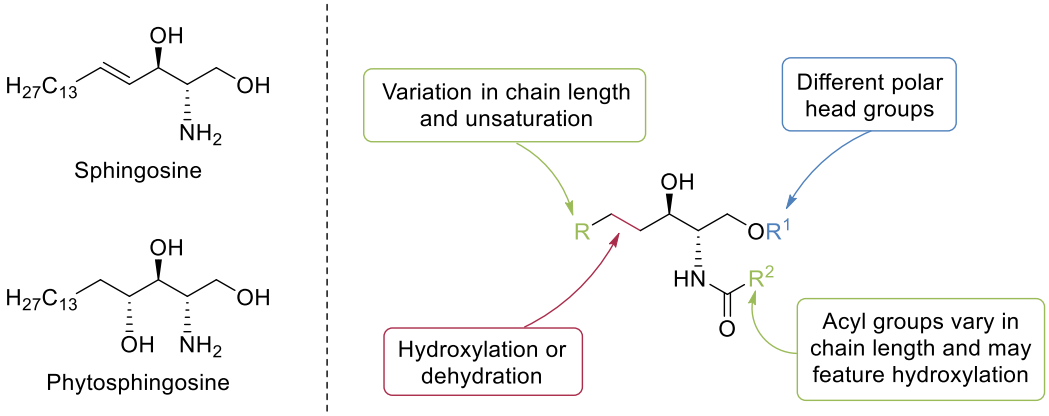
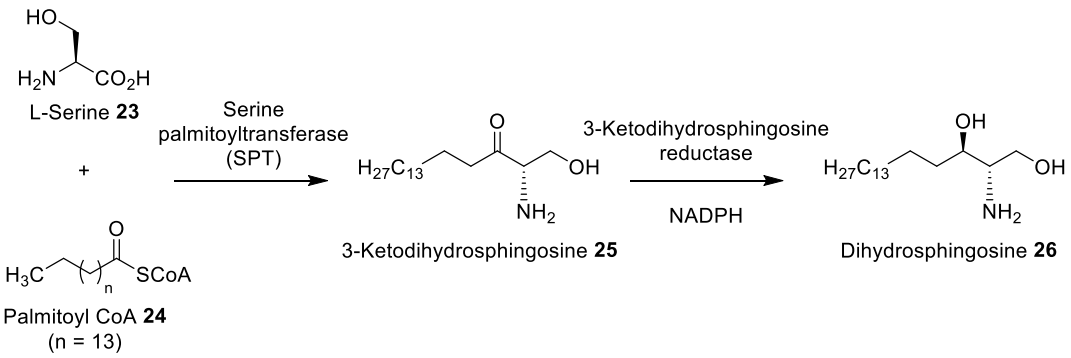


Figure 1–10: Variation in sphingoid bases and sphingolipids

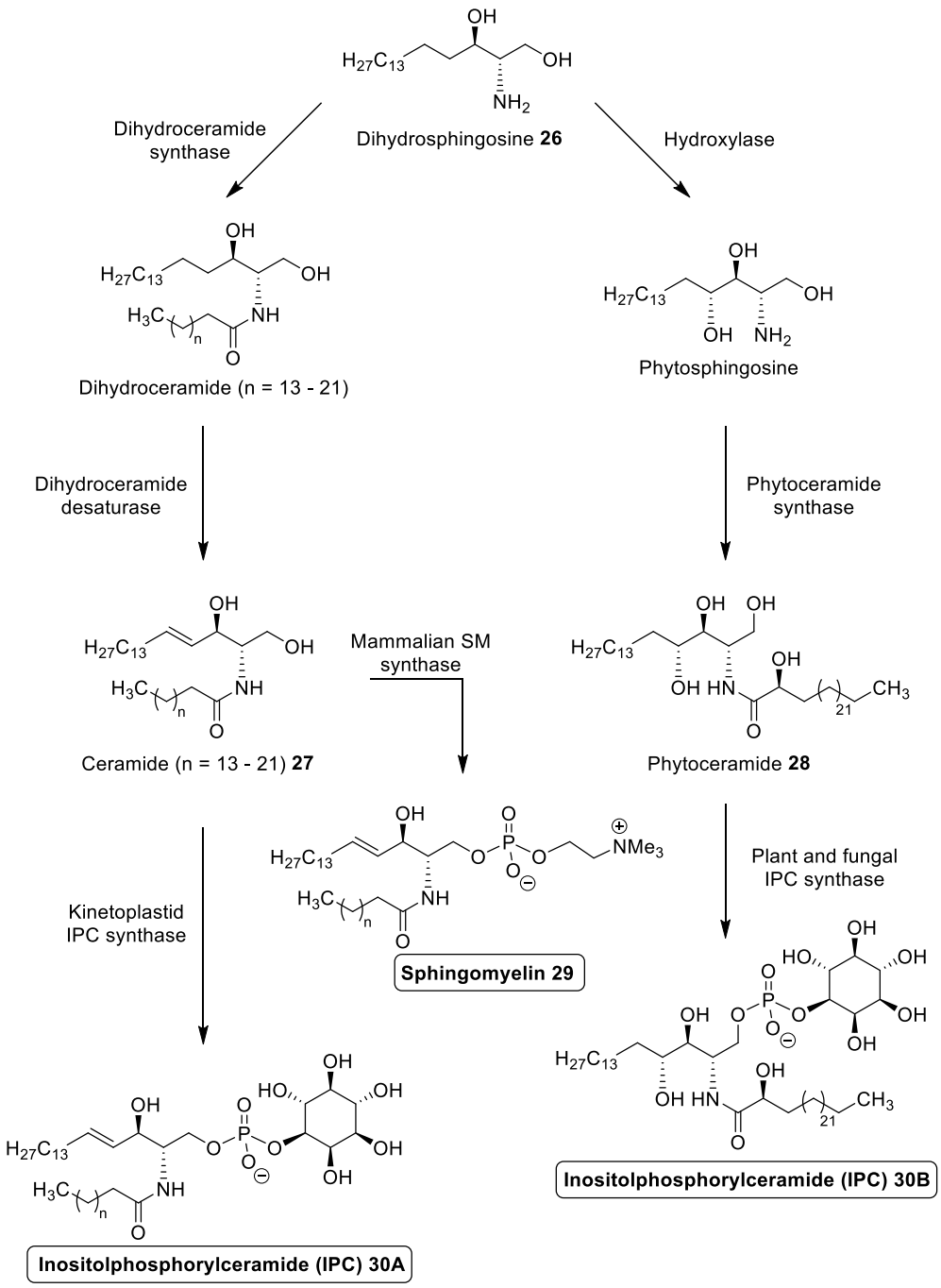
1.3.2 *De novo* synthesis

The biochemical synthesis of SLs in eukaryotes begins with the rate-limiting condensation of L-serine **23** and palmitoyl CoA **24** to form 3-ketodihydrosphingosine **25**, a reaction catalysed by serine palmitoyltransferase (SPT) (Scheme 1–1).[156] Following reduction to dihydrosphingosine **26** by 3-ketodihydrosphingosine reductase and NADPH, divergence in the metabolic pathway is encountered.[156,157]



Scheme 1–1: Conserved sphingolipid biosynthetic pathway

In mammals, dihydrosphingosine **26** is subjected to *N*-acylation followed by unsaturation to afford ceramide **27**, whereas in fungi and higher plants, a hydroxylation step with successive *N*-acylation forms phytoceramide **28** (Scheme 1–2).[156] Subsequently, these simple sphingolipids are transported from the endoplasmic reticulum to the Golgi apparatus, where they may undergo further biosynthetic transformations. In mammalian cells, attachment of a choline head group to ceramide **27** *via* a phosphate ester linkage gives the principal sphingolipid sphingomyelin (SM) **29**, a process catalysed by sphingomyelin synthase (SMS).[156] In contrast, fungi and higher plants use inositol phosphorylceramide synthase (IPCS) to append a phosphoinositol head group onto phytoceramide **28**, furnishing inositol phosphorylceramide (IPC) **30B**.[156] Kinetoplastids, such as *Leishmania*, also form their principal sphingolipid **30A** *via* IPC synthase.[158,159] Interestingly, despite having separate functions, the human SM and protozoan IPC synthases both utilise ceramide **27** as a common substrate.



Scheme 1–2: Divergent sphingolipid biosynthetic pathways. IPC refers to the general class of complex sphingolipids containing the inositol sugar group. For clarity, the kinetoplastid and plant/fungal IPCs discussed in this chapter are labelled 30A and 30B respectively.

1.3.3 Sphingolipid metabolism and function in *Leishmania*

While sphingolipid biosynthesis in *Leishmania* has not been studied in its entirety, the majority of efforts have focussed on characterising the first step of *de novo* synthesis, catalysed by SPT,

and IPC synthesis. The discoveries made will be discussed below, including an evaluation of the SL biosynthetic pathway as a therapeutic target.

1.3.3.1 Serine palmitoyl transferase (SPT)

Studies by the Denny and Beverley groups found that SPT in *L. major* promastigotes is fully functional during log-phase growth of the procyclic form, but is gradually downregulated during metacyclogenesis, when the parasite differentiates to its metacyclic form.[160–162] Furthermore, SPT knock-out (*spt2*$^-$) mutant *L. major* promatigotes, generated by ablation of the *SPT2* subunit gene, were found to be viable in the procyclic stage, but unable to conduct metacyclogenesis.[160,161] This observation suggests that *de novo* SL synthesis is necessary for differentiation in promastigotes.[160] Indeed, SL-free *spt2*$^-$ *L. major* promastigotes exhibited poor infectivity against macrophages and mice, reflecting an inability to form infective metacyclic parasites.[160,161] Interestingly, SPT was found to be inactive in *L. major* amastigotes, and intracellular *spt2*$^-$ amastigotes were able to maintain their virulence *in vitro* and in mice. This implies that *de novo* SL synthesis is important for initial infection by metacyclic promastigotes, but is not essential for pathogenesis within macrophages.[160,162] By contrast, preliminary metabolomics and proteomics work on *L. mexicana* showed that *Lmx*SPT is not downregulated in amastigotes, although the implications of this have yet to be revealed.[163,164]

Myriocin **31** (a potent SPT inhibitor) and fumonisin B_1 **32** (an inhibitor of dihydroceramide synthase) do not inhibit proliferation of *L. major* promastigotes or intracellular amastigotes (Figure 1–11).[162] Similarly, promastigotes and amastigotes of *L. mexicana* prove resistant to myriocin **31**,[163] although fumonisin B_1 **32** inhibits replication of intramacrophage *L. donovani*.[165] Recently, myriocin **31** was found to inhibit sphingolipid synthesis in *L. braziliensis* promastigotes, disrupting growth and cytokinesis.[166] This differential activity of SL biosynthesis inhibitors across Old and New World *Leishmania* species is indicative of complex interactions which require further analysis.

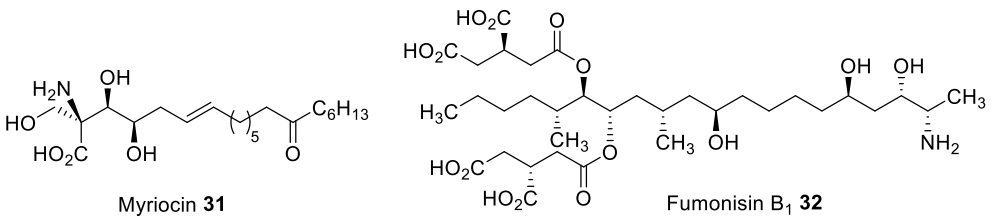


Figure 1–11: Inhibitors of early sphingolipid biosynthesis

1.3.3.2 Inositol phosphorylceramide synthase (IPCS)

Inositol phosphorylceramide **30A** (IPC) is the principal complex sphingolipid of *Leishmania*, accounting for 5 – 10% of total cellular lipids,[158] comprising a key component of detergent resistant membranes (DRMs), or lipid rafts, in *Leishmania* promastigotes.[167–169] Both wild type (WT) and *spt2*$^{-}$ *L. major* amastigotes, in which *de novo* SL synthesis is absent, were discovered to have an active IPCS enzyme, using salvaged host SL metabolites to produce IPC **30A** at levels comparable to those observed in WT promastigotes.[162] It is worth noting that phagocytised *L. donovani* upregulate the production of host ceramide **27**, which may act as a feedstock for IPC **30A** synthesis.[170] The fact that *L. major* amastigotes maintain high levels of IPC **30A** may indicate that this complex SL is required for survival within host macrophages. Indeed, remodelled host sphingolipids were implicated in the maintenance of acidocalcisomes in *L. major* promastigotes and amastigotes.[162] Acidocalcisomes are organelles which have been found to be important for stress responses and growth in the related protozoa *Trypanosoma cruzi* and *Trypanosoma brucei*.[171,172] Thus, targeting the synthesis of IPC **30A** in *Leishmania* may have therapeutic potential.

Aureobasidin A (AbA) **33**, a potent fungal IPCS inhibitor,[173] has been tested against promastigotes of *L. amazonensis*, *L. braziliensis* and *L. major*, exhibiting ED_{50} values of 4.1 µM, 13.7 µM and 12.6 µM respectively (Figure 1–12).[168,174] However, the action of AbA **33** against *Leishmania* does not seem to be caused by IPCS inhibition. Denny found AbA **33** to be effective against mutant *spt2*$^{-}$ *L. major* promastigotes, where *de novo* SL synthesis is absent, rendering IPCS activity redundant.[159] A further experiment against *Leishmania major* IPCS, expressed in a mutant yeast system, found that AbA **33** has no effect until 100 µM, confirming that AbA **33** acts against *Leishmania* through a different, unknown mechanism.[159] This data highlights a significant difference between the IPC synthases of *Leishmania* and yeast.

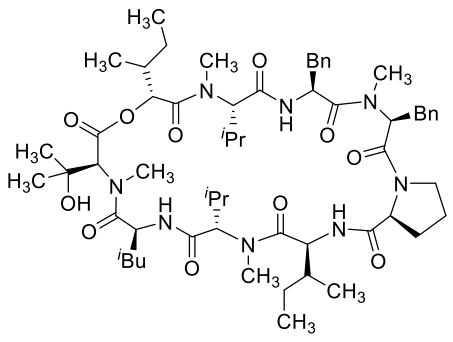


Figure 1–12: Aureobasidin A 33, a potent fungal IPCS inhibitor

1.3.3.3 Sphingolipid degradation

Studies by Zhang on SL degradation revealed the presence of a homolog of human sphingomyelinase (SMase) in *L. major*.[175] This inositol phosphosphingolipid phospholipase C-like (ISCL) protein was found to have dual SMase and IPCase activity, presumably for the breakdown of both host salvaged SM **29** and endogenous IPC **30A**. ISCL-null promastigote mutants (*iscl*$^{-}$) proliferated in a normal manner *in vitro*, but failed to proliferate or cause pathology in immunocompetent or even immunodeficient mice.[176] *Iscl*$^{-}$ *L. major* promastigotes were also found to be vulnerable in acidic medium (pH 5), explaining their inability to survive within the acidic phagolysosomes of murine macrophages.[176] Further work showed that the breakdown of IPC **30A** conferred acid resistance to promastigotes during initial infection, but SM **29** degradation was responsible for enabling virulence and proliferation of amastigotes in mice.[177]

ISCL in *L. amazonensis* promastigotes was found to have an expression profile and biochemical properties similar to its ortholog in *L. major*.[178] Mutants lacking *La*ISCL accumulated IPC **30A**, and could still undergo transformation to axenic amastigotes, but, as expected, had lower survival levels in acidic (pH 5.3) media. Unlike *L. major iscl*$^{-}$ parasites, however, *L. amazonensis* mutants were fully infective against BALB/c mice.[178]

Zhang *et al.* investigated another enzyme involved in SL degradation, sphingosine-1-phosphate lyase (SPL).[179] A study of *spl*$^{-}$ mutant *L. major* promastigotes exhibited phenotypes similar to those observed in SL free *spt2*$^{-}$ parasites. Ethanolamine was found to reverse the viability and differentiation defects of these mutants; it was put forward that an important role of SL synthesis and degradation in *Leishmania* is the production of ethanolamine phosphate, a metabolite essential for metacyclogenesis.[179] However, in myriocin **31** treated *L. braziliensis*, defects in growth and cytokinesis were not rescued by ethanolamine, but rather the addition of 3-ketodihydrosphingosine **25**.[166] Furthermore, ISCL activity does not seem to be related to ethanolamine production,[175] which may relate to the observation that *L. major* amastigotes either do not require ethanolamine, or scavenge it from host macrophages.[179] Thus, while SL metabolism may be important for ethanolamine production in *L. major* promastigotes, it does not seem to fill this role for amastigotes or other *Leishmania* species.

1.3.3.4 *Leishmania* SL synthesis as a therapeutic target

Although the full role of sphingolipid synthesis in *Leishmania* has yet to be realised, it remains that metabolites along the biosynthetic pathway must maintain a fine balance. For example, preliminary work on a sphingosine kinase (SK) in *L. major* revealed that *ska*$^{-}$ knock out mutants, lacking the sphingosine kinase A enzyme, had compromised viability due to toxic accumulation of sphingoid bases and susceptibility to the host immune system.[180] Ceramide **27** is well known for its function in apoptosis (programmed cell death).[181–184] Reports detailing the modulation of ceramide **27** activity by sphingosine-1-phosphate and diacylglycerol (DAG) species highlight the delicate balance of cell growth and death.[185,186] More specifically, DAG produced by sphingomyelin synthase activity is important for the proliferation of mammalian cells.[187–189] Thus, interfering with intracellular levels of ceramide **27** and DAG, respective substrate and by-product of IPCS, in *Leishmania* can potentially trigger apoptosis, whilst starving the parasite of mitogenic DAG. The *Leishmania* IPCS therefore represents an attractive drug target.[159,190] Furthermore, the differential function of human SMS opens up an opportunity to develop specific inhibitors of *Leishmania* IPCS which will have low host toxicity.[159]

1.3.4 *Leishmania major* IPCS

1.3.4.1 Precedent of fungal IPCS as a drug target

In pathogenic fungi, IPCS, encoded by the *AUR1* gene, has been investigated as a viable therapeutic target, due to the lack of a mammalian equivalent.[191,192] The enzyme, located within the Golgi apparatus,[193] was found to be essential for survival, as mutants of *Saccharomyces cerevisiae* lacking IPCS accumulated ceramide, leading to cell death.[192] Furthermore, mutagenesis of the catalytic triad (two histidines and one aspartate) inactivated the enzyme and prohibited cell growth.[193] Of particular note is the discovery that IPCS is required for virulence and growth of *Cryptococcus neoformans*, a pathogenic yeast which, like *Leishmania*, resides inside host phagolysosomes.[194] A number of IPCS inhibitors have been described that demonstrate potent antifungal activity.[173,195,196]

1.3.4.2 Discovery of *Lmj*IPCS

Denny *et al*. identified the single copy gene encoding the functional *AUR1* orthologue from *L. major* (*Lmj*IPCS, *Lmj*F35.4990) through bioinformatics and functional genetic methods.[159]

Phylogenetic analysis defined *Lmj*IPCS as belonging to a class of kinetoplastid SL synthases distinct from fungal and mammalian orthologues.

Leishmania major IPCS is a membrane bound protein, localised within the Golgi apparatus, comprised of 338 amino acids with a mass of approximately 38 kDa (Figure 1–13). The enzyme contains a putative catalytic triad of conserved histidine and aspartate residues, as defined in the orthologous yeast IPCS and animal SMS enzymes.[197,198] As *Lmj*IPCS is an integral membrane protein, traditional structure elucidation methods, such as protein crystallography, remain a significant challenge. Thus, the true structure of *Lmj*IPCS remains unknown. However, a hypothetical topology model was proposed by Sutterwala *et al.*, based on alignment of kinetoplastid SLS sequences with a model for human SMS2 (Figure 1–13).[198,199] Interestingly, despite possessing functional similarity to fungal IPC synthases, *Lmj*IPCS shares greater similarity with mammalian SMS enzymes, perhaps due to the requirement for ceramide **27** as a substrate.[159,199]

1	MTSHVTAHDV	GGNEDIGTDH	VPWYKQPLPL	CTQVMRFILL	LLLTVMFLGV
51	AILVANARMP	DPEKVRPLPD	LLLESIPKVA	LLENGTNVII	FLLNATTVVV
101	GFKVFLLERH	MNGLPRVTFL	VGVPKIGSFL	NRMAFGVLDS	GRRPFPLKNV
151	FPIMAIRFLT	SYAVVMVFRA	FVIMGTSYPA	TDNHCQNPQV	IEHPVLNVIL
201	TLVTLGSGAI	HCGDLMFSG***H***	TMILSLAFIL	AWDYSPFLHP	WAVRVWVSVL
251	LPISYYCILA	SRS***H***YTD***D***IL	VAMYVMIATY	KVIDHAETGA	PWQMQLLIRW
301	MPWPGANTIE	KWTADEVVVV	VQTPAEDSTD	ASAALPEH	

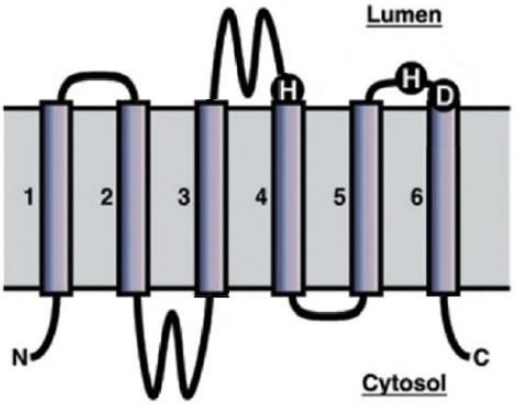


Figure 1–13: Top: *Lmj*IPCS protein sequence, with highlighted catalytic triad; Bottom: Bangs' theoretical topology of kinetoplastid SLS with highlighted catalytic triad. Reprinted with permission.[199]

1.3.4.3 Genetic validation of *Lmj*IPCS

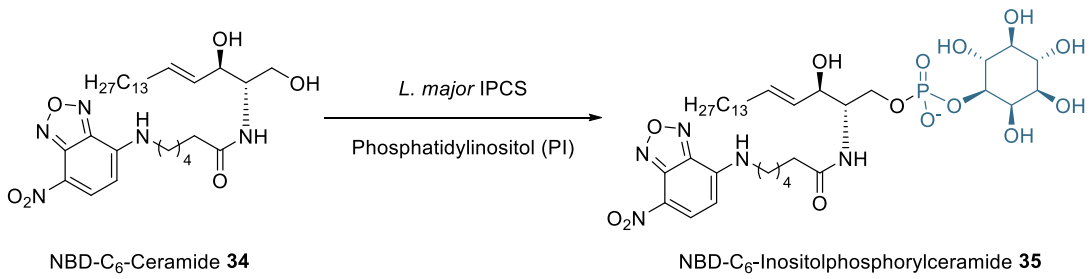
Methods for genetic manipulation of *Leishmania* suffer from a number of limitations. *Leishmania* is an asexual diploid, hindering forward and reverse genetic methods and requiring two rounds of gene targeting to generate null mutants.[200,201] Further complications arise from the tendency of *Leishmania* towards 'mosaic aneuploidy',[202,203] meaning additional copies of essential genes may still be found, even after two rounds of targeting.[204]

Utilisation of RNA interference (RNAi), a popular method of supressing gene expression, is not viable for Old World species, such as *L. major* and *L. donovani*, as they lack a functional RNAi pathway.[111,200] Interestingly, however, New World *L. braziliensis* possesses a full complement of RNAi pathway genes;[205,206] the source of this incongruity between *Leishmania* species remains unclear.[207]

Denny *et al.* sought to create *ipcs*$^-$ mutant *L. major* through homologous recombination. This did not prove to be possible with WT promastigotes, presumably due to an accumulation of toxic metabolites (data unpublished). Attention then turned towards targeting *Lmj*IPCS in *spt2*$^-$ *L. major*, where the lack of *de novo* SL synthesis would ensure no lethal build-up of biosynthetic intermediates. Removal of the first *Lmj*IPCS chromosomal gene was facile, but upon attempting removal of the second gene copy it became apparent that the parasite avoided loss of the enzyme by translocation of the coding region (data unpublished). This phenomenon has previously been observed for other essential genes.[208] Thus, a *Leishmania major* mutant lacking a functional IPC synthase remains elusive. Nevertheless, the sphingolipid deficient *spt2*$^-$ *L. major* mutant may serve as a proxy to test the selectivity of potential IPCS inhibitors, as the *Lmj*IPCS enzyme is redundant in these parasites.

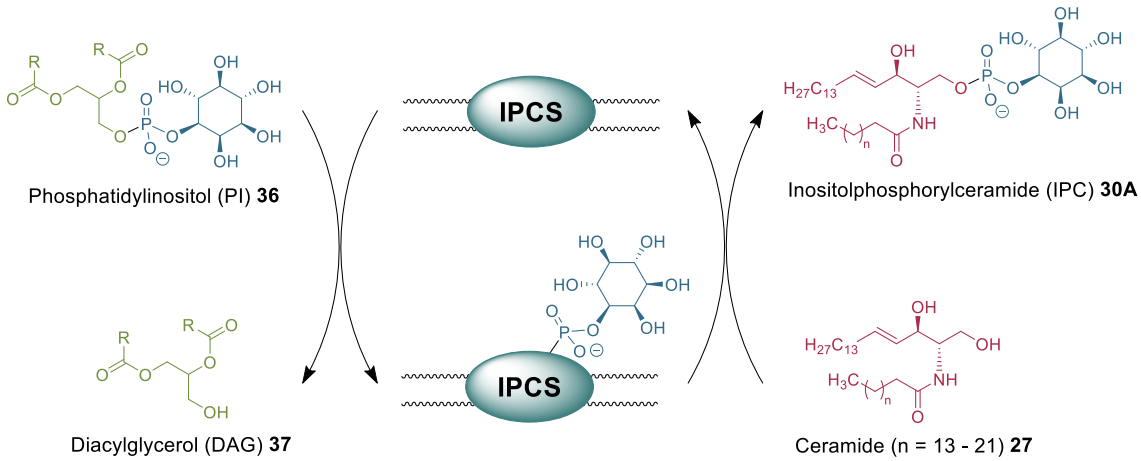
1.3.4.4 Characterisation of *Lmj*IPCS

Mina characterised the *Leishmania major* IPC synthase enzyme, through development of a cell-free microtiter plate compatible assay, adapted from a method used for the study of the orthologous *Candida albicans* AUR1p enzyme (Scheme 1–3).[209,210] Firstly, a mutant *S. cerevisiae* yeast strain (YPH499–HIS–GAL–AUR1 pRS246–*Lmj*IPCS), under which *Sc*AUR1 production is repressed in the presence of glucose,[159] was used for the isolation of microsomal membranes enriched in *Lmj*IPCS.[209] This microsomal material was used to measure the conversion of fluorescent NBD-C_6-ceramide **34** to NBD-C_6-IPC **35**, with the respective substrate and product being separated by anion exchange chromatography prior to fluorescence spectroscopy.



Scheme 1–3: *Lmj*IPCS biochemical assay (see Section 9.4 for further information)

Using this method, the kinetic parameters of *Lmj*IPCS were examined, showing the enzyme function to occur *via* a bi-bi (ping-pong) model with double displacement kinetics (Scheme 1–4).[209] Furthermore, ceramide **27** was found to have a higher affinity for the enzyme than phosphatidylinositol (PI) **36**; which, when taken into account with the observation that PI **36** is relatively abundant, suggests that ceramide **27** is the rate-limiting substrate in this reaction.[158,209]



Scheme 1–4: Catalytic cycle of *Lmj*IPCS

Further work by Mina centred on probing the ceramide binding domain of *Lmj*IPCS through synthesis of a number of ceramide analogues.[190] Utilising the 96-well plate compatible assay introduced above (Scheme 1–3), coupled with mass spectrometry analysis, a number of structure-activity relationships (SARs) were uncovered (Figure 1–14). Additionally, ceramide analogue **38** demonstrated cytotoxicity against *L. major* promastigotes, including modest selectivity over the SL free *spt2*$^{-}$ mutant, serving as chemical validation of *Lmj*IPCS as a viable target.[190]

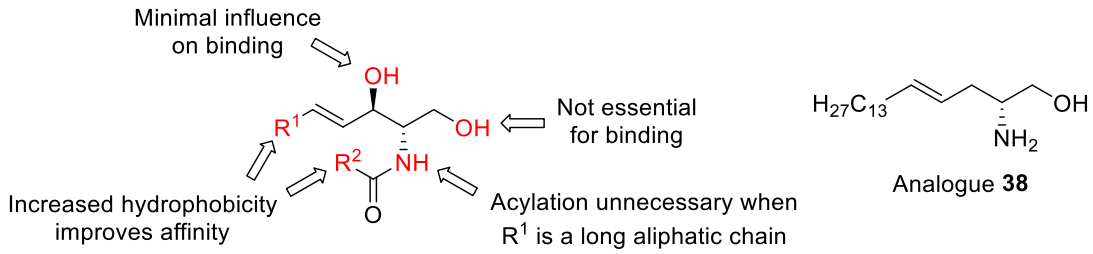


Figure 1–14: SAR of ceramide analogues and free amine derivative 38

Similarities between this work and reported activity of sphingosine analogues against a lipid phosphate phosphatase (LPP),[211] combined with the observation of conserved catalytic residues between LPP and SLS enzymes,[159,198] point towards a common mechanism of action. Sigal *et al.* proposed a general mechanism for phosphoryl transferase enzymes, which include LPPs and SLSs, which was adapted by Mina *et al.* to propose a putative mechanism of action for *Lmj*IPCS (Figure 1–15).[190,212] Of particular note is the conserved Arg262 residue, which is assumed to be protonated to provide electrostatic stabilisation of the negatively charged transition state. It was postulated that the ammonium salt of sphingosine analogue **38** may interfere with this interaction, giving rise to the observed IPCS inhibition.[190]

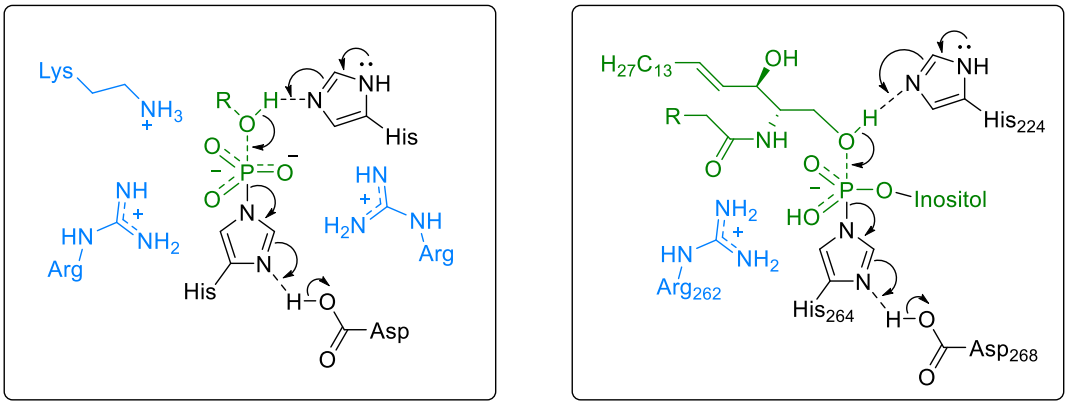


Figure 1–15: Left: Sigal's proposed general mechanism of lipid phosphatases;[212] Right: Sigal's model applied to the putative mechanism of *Lmj*IPCS

1.4 Previous work within the group

Utilising the assay described above (Scheme 1–3),[209] a 1040 member set of pharmacologically active compounds* was screened at 20 µM against the *Lmj*IPCS enzyme (data unpublished) (Figure 1–16). Fifty-seven compounds were found to exhibit >70% inhibition of the IPC

* National Institute of Neurological Disorders and Stroke (NINDS) library, supplied by Medical Research Council Technology (MRCT) Ltd.

synthase; analysis of Z-factor values confirmed that all data obtained was of excellent quality.[213]

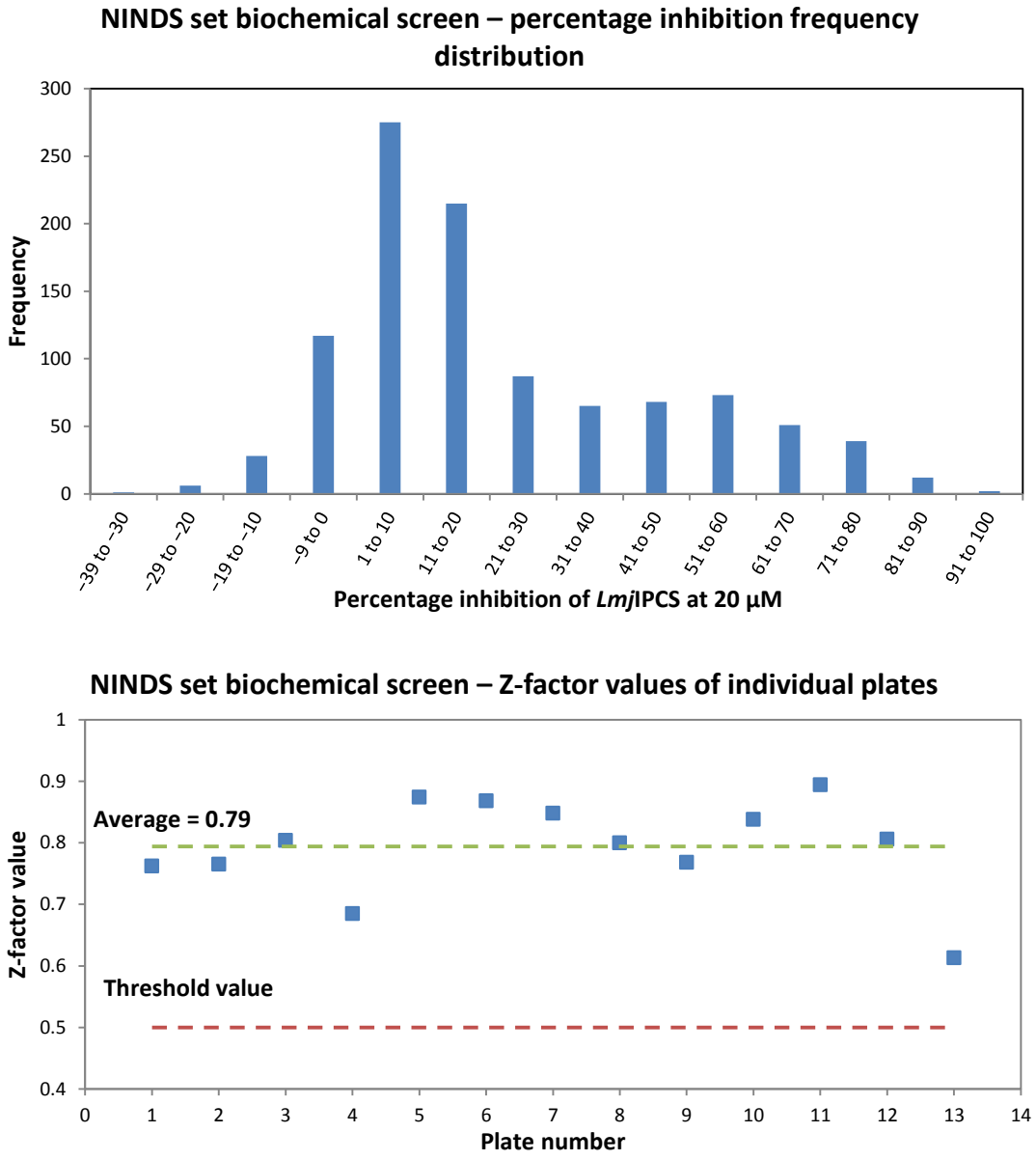


Figure 1–16: Top: Primary biochemical screen of NINDS set against *Lmj*IPCS; Bottom: Calculation of Z-factor values as a measure of data quality.

A second round of screening was conducted, in which these 57 inhibitors were tested for cytotoxicity against *L. major* promastigotes at 10 µM (data unpublished) (Figure 1–17). Using a resazurin-based cell-viability assay,[214] the majority of compounds were found to inhibit promastigote growth after 24 hours. In order to assess the difference between cytostatic and cytotoxic agents, the assay was repeated with a post-dosage incubation of 72 hours. As expected, the activity of most compounds diminished, leaving known cytotoxic agents (red

bars), the control amphotericin B **3** (lane 59) and a number of new inhibitors (green bars). Following removal of the known cytotoxic compounds, included in the library as controls, 16 new inhibitors were identified as potential lead structures. These represented the starting point for the work described in the following chapter.

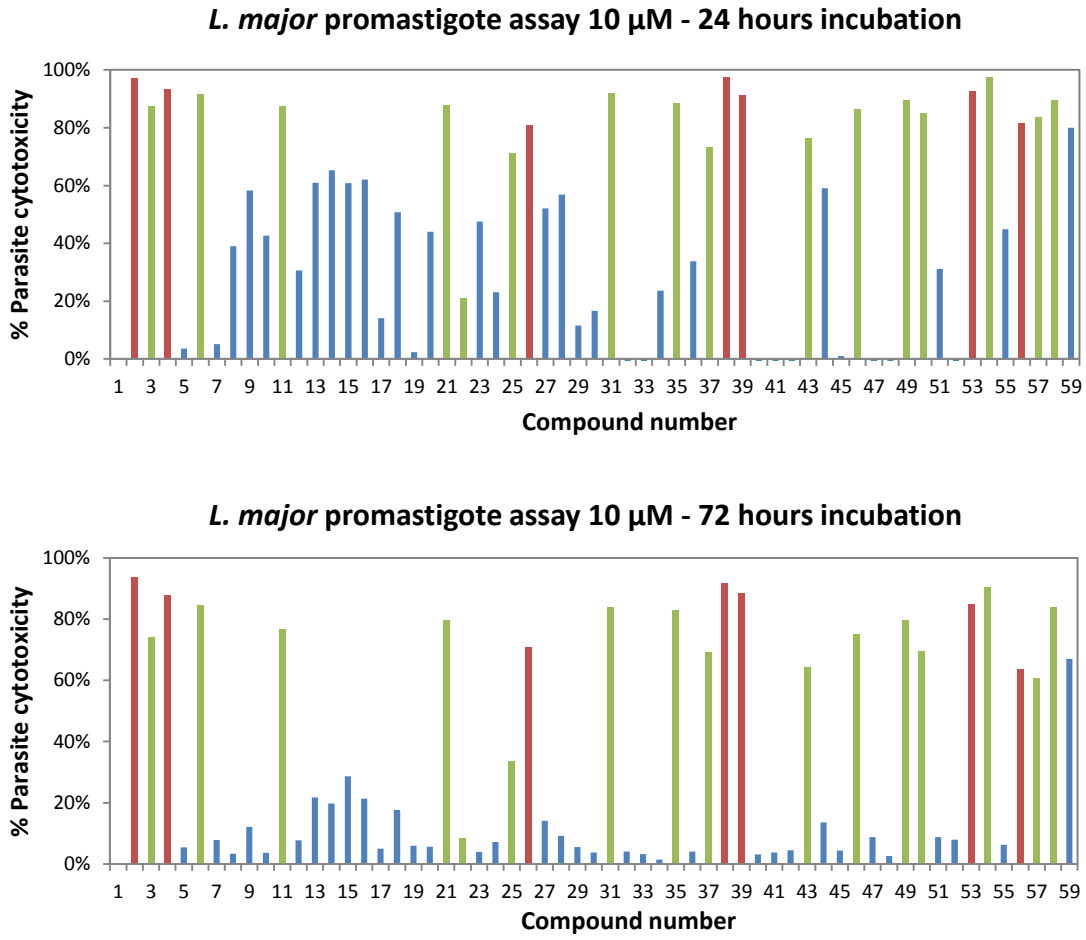


Figure 1–17: Cytotoxicity of compounds from NINDS library against *L. major* promastigotes. Top: 24 hrs post dosage and Bottom: 72 hrs post dosage with compounds. (Lane 1: no inhibitor; Lane 59: amphotericin B control; Lanes 2, 4, 26, 38, 39, 53 and 56 (red bars) are known non-selective cytotoxic compounds; Lane 22 is the known antileishmanial pentamidine 5).

1.5 Project objectives

In light of the preliminary data obtained from the NINDS library screen, the first objective was to continue analyses of the 16 chosen inhibitors. Confirmation of hit compound activity would be carried out, prior to a challenge against the clinically relevant amastigote form of *Leishmania major*.

Subsequent work would then focus on chemical elaboration of the most promising hit(s) from the NINDS set, with a view to generating SAR data to probe the *Leishmania major* IPCS enzyme. This data would inform the synthesis of a second generation of *Lmj*IPCS inhibitors with leishmanicidal action.

2. NINDS Library Screen

The results within this chapter comprise work carried out by the Denny group. Other than the exceptions detailed in Table 2–1 and Figure 2–2, all work is the author's own. The experiments in Section 2.2 were undertaken by the author at the London School of Hygiene and Tropical Medicine, with guidance kindly provided by Dr Yardley and Professor Croft.

2.1 Confirmation of hit compound activity

The screening data, and structures, of the 16 compounds identified *via* the efforts discussed in Section 1.4 are displayed below (Table 2–1 and Figure 2–1).

Table 2–1: Preliminary screening data from the chosen NINDS library hits, carried out by J.G.M. Mina with compounds supplied by Medical Research Council Technology Ltd

Compound	*Lmj*IPCS inhibition (20 µM)	*L. major* inhibition 72 h (10 µM)
Amlodipine **39**	80%	62%
Chlorpromazine **40**	88%	76%
Clemastine **41**	83%	61%
Clomiphene **42**	76%	65%
Doxepin **43**	74%	44%
Fendiline **44**	71%	74%
Flunarizine **45**	74%	56%
Pararosaniline **46**	78%	75%
Pentamidine **5**	86%	6%
Pimozide **47**	77%	56%
Prochlorperazine **48**	83%	11%
(±)-Propranolol **49**	73%	84%
Sertraline **50**	78%	48%
Sulfamethizole **51**	77%	68%
Suloctidil **52**	81%	74%
Trifluoperazine **53**	86%	67%

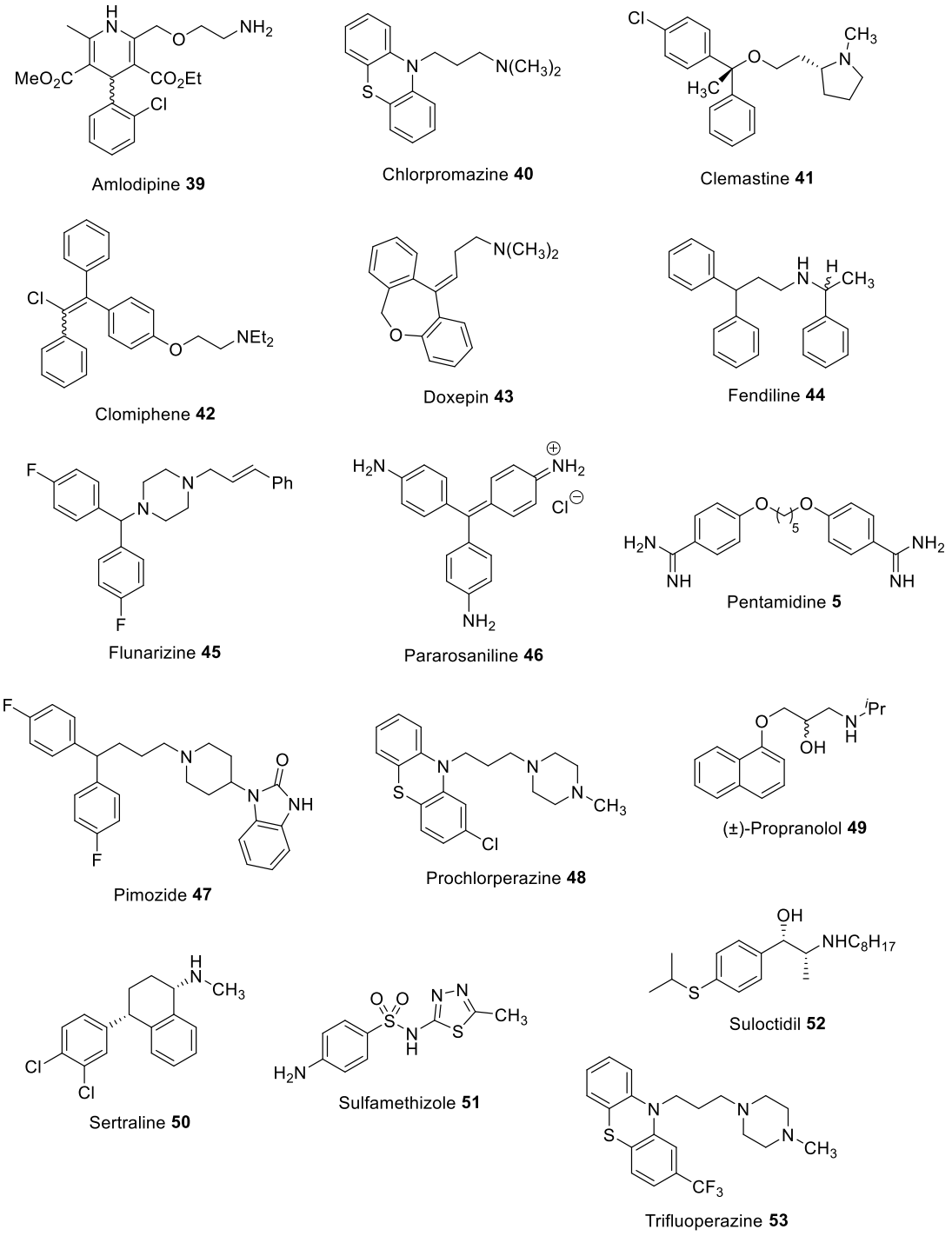


Figure 2–1: NINDS library hit compounds selected for further study

The antileishmanial activity of the structurally similar tricyclic compounds prochlorperazine **48** (11%), chlorpromazine **40** (76%) and trifluoperazine **53** (67%), at 10 µM, were found to differ significantly (Table 2–1). It was postulated that the observed difference either reflected a genuine structure-activity relationship (SAR) finding, or was a discrepancy caused by the plausible presence of degradation products in the supplied DMSO solutions. Indeed, this phenomenon has been previously reported with prolonged storage of DMSO solutions.[215]

Furthermore, this latter hypothesis was corroborated by the fact that the supplied pentamidine **5**, a known antileishmanial drug, exhibited uncharacteristically low parasiticidal activity (6% after 72 h incubation).

In order to confirm the activity observed with the chosen hit compounds, pure material from commercial sources was used in a dose-response study against *Leishmania major* IPCS and promastigote parasites (Figure 2–2). IC_{50} values could not be determined for pararosaniline **46** and sulfamethizole **51**, which also exhibited poor antileishmanial activity (ED_{50} = 9.78 µM and 39.6 µM respectively). Interestingly, whilst IPCS inhibition was confirmed for doxepin **43** and pimozide **47**, these compounds displayed ED_{50} values significantly higher than 10 µM against *L. major* promastigotes (30.0 µM and 22.9 µM respectively). These observations disagree with the inhibition values observed in the initial NINDS library screen (*c.f.* Table 2–1), plausibly due to the presence of degraded material.

The tricyclic compounds chlorpromazine **40**, prochlorperazine **48** and trifluoperazine **53** all displayed similar inhibitory effects against *L. major* IPCS (Figure 2–2). Furthermore, no significant difference was found between the antileishmanial activity of prochlorperazine **48** (ED_{50} = 12.9 µM, 95% CI: 11.3 – 14.7 µM) and that of chlorpromazine **40** (ED_{50} = 9.80 µM, 95% CI: 6.94 – 13.9 µM), indicating that the disparate values in Table 2–1 may be due to error or compound degradation. It is worth noting, however, that the leishmanicidal activity of trifluoperazine **53** (ED_{50} = 7.17 µM, 95% CI: 6.28 – 8.19 µM) was found to be nearly double that of prochlorperazine **48**. This highlights an interesting structure-activity relationship for these tricyclic inhibitors: *meta*-trifluoromethylation is preferred over *meta*-chlorination for antileishmanial activity.

Pentamidine **5** was confirmed as an inhibitor of *Lmj*IPCS, displaying an IC_{50} value of 3.10 µM (95% CI: 2.20 – 4.35 µM). Moreover, the comparatively lower ED_{50} value (2.05 µM, 95% CI: 1.34 – 3.00 µM), obtained against *L. major* promastigotes, reflects the additional known modes of action of pentamidine **5** (see Table 1–1).

Comparatively, the compounds clomiphene **42**, fendiline **44**, (±)-propranolol **49** and sertraline **50** were all found to display moderate biochemical and antileishmanial activity. In addition, for the individual compounds, there do not seem to be any notable disparities between the data in Table 2–1 and Figure 2–2. For these reasons, the compounds were not studied further.

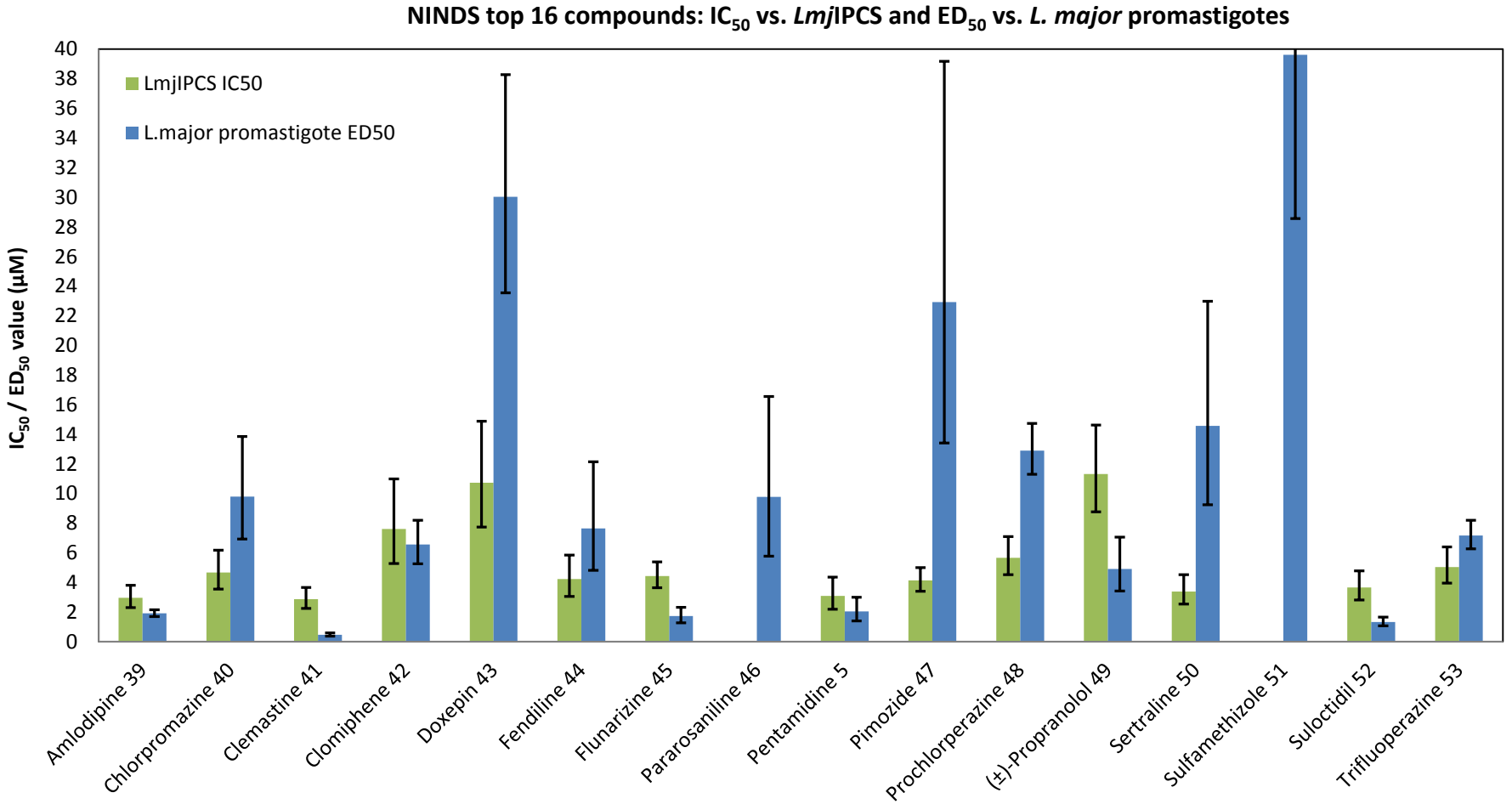


Figure 2–2: Comparison of IC_{50} vs. *Lmj*IPCS and ED_{50} vs. *L. major* promastigotes. Error bars portray 95% confidence interval. IC_{50} data was gathered by the author. ED_{50} data was gathered by J.G.M. Mina.

Four compounds exhibited antileishmanial activity exceeding that of pentamidine **5** (Figure 2–2). Amlodipine **39** (ED_{50} = 1.91 µM, 95% CI: 1.70 – 2.16 µM), clemastine **41** (ED_{50} = 0.47 µM, 95% CI: 0.37 – 0.59 µM), flunarizine **45** (ED_{50} = 1.73 µM, 95% CI: 1.28 – 2.33 µM) and suloctidil **52** (ED_{50} = 1.33 µM, 95% CI: 1.07 – 1.66 µM) were all selected for further investigation. Interestingly, these compounds were found to have higher potency against promastigotes than the *Lmj*IPCS enzyme. This phenomenon may be attributed to multiple factors, for example: activity against more than one enzyme (pleiotropism); increased concentration around the Golgi apparatus where *Lmj*IPCS is predicted to be localised;[159] or conversion of compounds to metabolites with enhanced cytotoxicity.

Overall, the majority of the 16 NINDS compounds that were retested were confirmed to have micromolar activity against the *Lmj*IPCS enzyme and *L. major* promastigote parasites. It is interesting to note that many of these compounds have structural similarity, in that they contain both an aryl-rich portion and an amine moiety, connected *via* an aliphatic linker. It was acknowledged that no significant SAR data could be drawn from the above results and hence further investigation would be required.

2.2 Activity against intramacrophage *L. major* amastigotes

The most effective leishmanicidal compounds, amlodipine **39**, clemastine **41**, flunarizine **45** and suloctidil **52**, were tested against murine peritoneal macrophages infected with *Leishmania major* amastigotes. Whilst flunarizine **45** only displayed activity at concentrations greater than 30 µM (data not shown), amlodipine **39** (ED_{50} = 3.65 µM, 95% CI: 3.42 – 3.89 µM), clemastine **41** (ED_{50} = 3.06 µM, 95% CI: 2.57 – 3.66 µM) and suloctidil **52** (ED_{50} = 4.28 µM, 95% CI: 3.64 – 5.04 µM) exhibited micromolar inhibition of parasite growth (Figure 2–3). Macrophage cytotoxicity was observed for concentrations of amlodipine **39** and suloctidil **52** over 10 µM, and clemastine **41** over 20 µM, hindering a more accurate assessment of activity. This cytotoxicity was confirmed against uninfected macrophages (Figure 2–3).

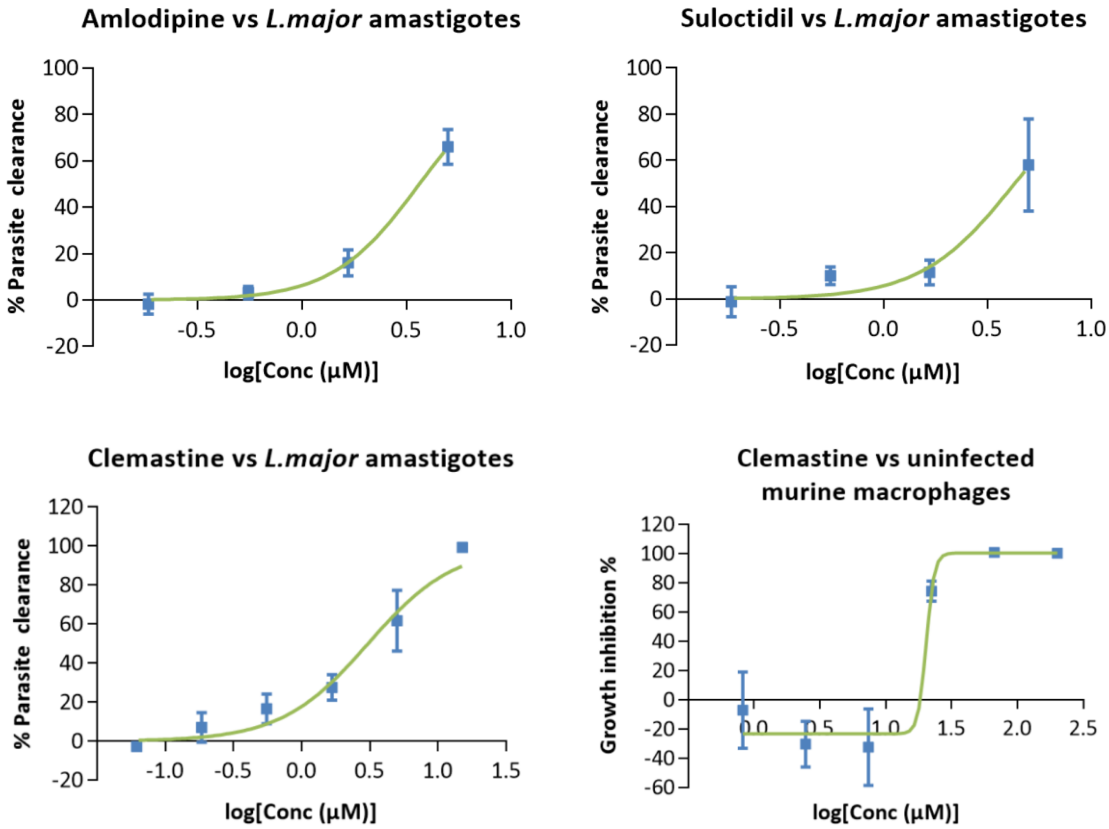


Figure 2–3: Activity against intramacrophage *L. major* and cytotoxicity of clemastine 41. Error bars portray 95% confidence interval.

Nevertheless, the *in vivo* safety profile of clemastine **41** is well documented,[216,217] with oral LD_{50} values of 730 $mg.kg^{-1}$ (mouse) and 3550 $mg.kg^{-1}$ (rat).[216] Furthermore, clemastine **41** displays low toxicity against human liver HepG2 cells ($ED_{50} > 50$ µM),[218] and low cardiotoxicity.[219] Due to its reported safety, and the observed potency against *L. major* promastigotes and clinically relevant amastigotes, clemastine **41** was advanced as the lead hit from the NINDS library screen.

2.3 Clemastine activity against *spt*$^-$ *L. major* mutant

To establish whether the antileishmanial action of clemastine **41** occurred *via* its activity as an inhibitor of *Lmj*IPCS, experiments were conducted with the sphingolipid-free *spt2*$^-$ *Leishmania major* mutant.[160] Whilst these parasites contain a functional IPC synthase, the lack of *de novo* synthesised ceramide renders IPCS activity redundant. The dose-dependent growth inhibition of *spt2*$^-$ *L. major* was assessed, revealing an ED_{50} of 8.65 µM (95% CI: 7.08 – 10.56 µM) and a selectivity index of ~20, compared to wild type promastigotes (Figure 2–4). By contrast, amphotericin B **3** maintained sub-micromolar activity against the mutant promastigotes

(ED_{50} = 0.15 µM, 95% CI: 0.08 – 0.27 µM against *spt2*$^{-}$; ED_{50} = 0.11 µM, 95% CI: 0.10 – 0.13 µM against WT), highlighting its separate mode of action (see Table 1–1).

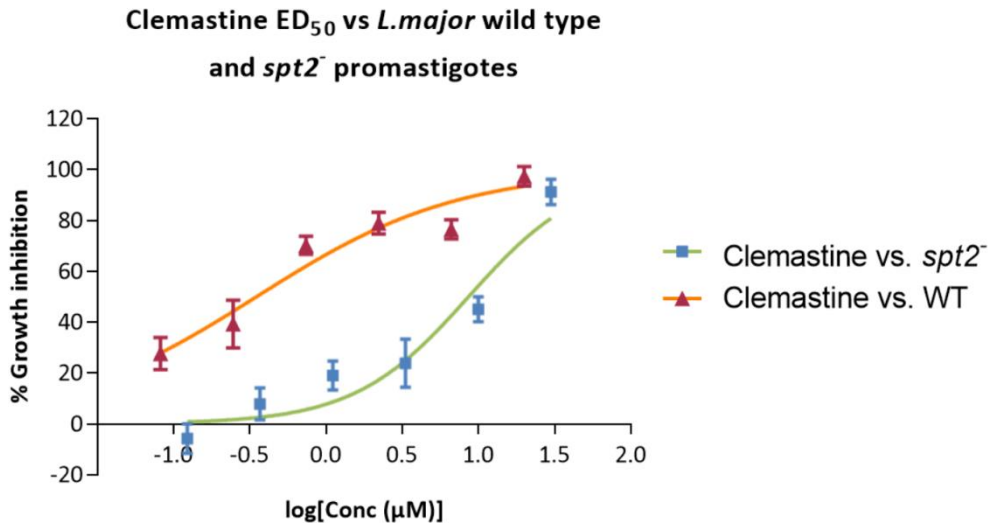


Figure 2–4: Activity of clemastine 41 against SL-deficient *L. major* promastigotes. Error bars portray 95% confidence interval.

The ability of clemastine **41** to inhibit IPCS *in cellulo* was measured in an experiment where *spt2*$^{-}$ *L. major* were incubated with clemastine **41** for 18 h prior to the addition of ceramide labelled with a BODIPY fluorophore (Figure 2–5). The use of this cell line ensured that IPCS activity relied solely on the addition of exogenous ceramide. Following cell lysis, fluorescent ceramide and IPC were separated *via* high performance thin layer chromatography (HPTLC) and quantified through fluorescence spectroscopy. Cells were incubated in media lacking foetal bovine serum (FBS) to ensure only fluorescent ceramide was metabolised by the active IPC synthase.

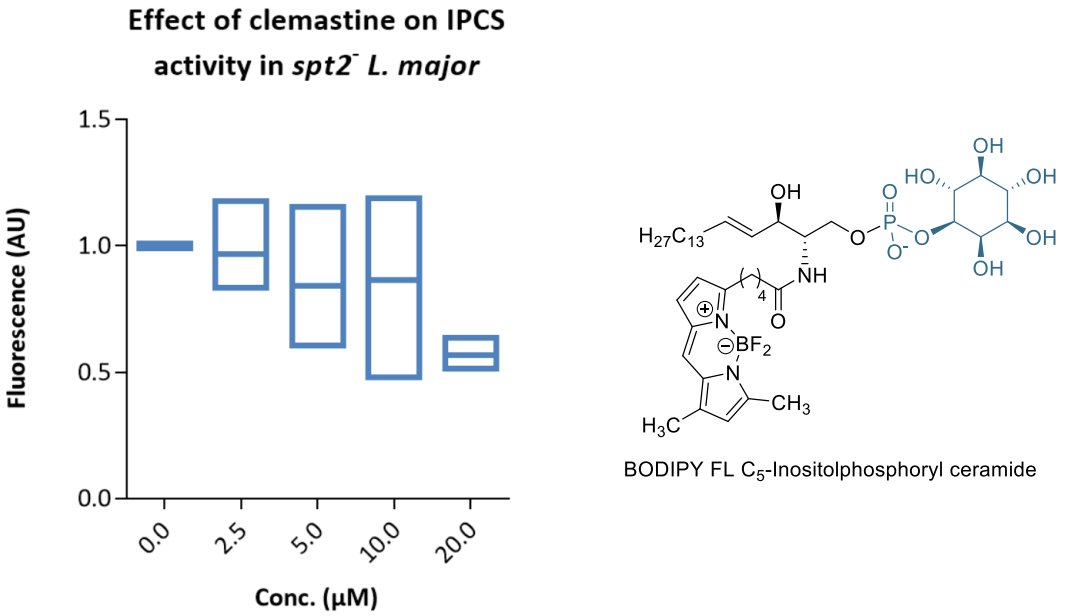


Figure 2–5: Clemastine 41 reduces BODIPY-IPC production in *spt*⁻ *L. major.* Mean values and range are shown. Values are normalised to IPC synthesis in the absence of clemastine 41.

A decrease in IPC synthesis was seen with increasing concentrations of clemastine **41**, consistent with *Lmj*IPCS inhibition. However, the data are not sufficient to calculate an accurate IC_{50} value (25.77 µM, 95% CI: 8.89 – 74.72 µM). Nevertheless, it is worth noting that *spt2*⁻ *L. major* promastigotes have a vastly different glycolipid composition compared to WT parasites,[160] manifesting as a thicker glycan surface coat and the accumulation of vesicles and glycosylated material around the Golgi.[160,162] This may significantly perturb the ability of clemastine **41** to enter cells and efficiently inhibit *Lmj*IPCS.

Overall, clemastine **41** was discovered to have significantly lower activity against *spt2*⁻ *L. major*, where IPCS is redundant, compared to wild-type promastigotes. Furthermore, the ability to inhibit IPC synthesis in a cellular context supports the hypothesis that this inhibition is responsible for the leishmanicidal effect of clemastine **41**, chemically validating *Lmj*IPCS as a viable target.

2.4 Conclusions

A primary screen of the NINDS compound library revealed a number of compounds with activity against *L. major* IPC synthase and promastigote parasites. The majority of these hits were confirmed using commercially sourced material, revealing four promising candidates: amlodipine **39**, clemastine **41**, flunarizine **45** and suloctidil **52**. Subsequent experiments against intramacrophage *L. major* amastigotes highlighted clemastine **41** as the most effective

compound. An investigation utilising sphingolipid-free (*spt2*$^{-}$) parasites confirmed the action of clemastine **41** as an inhibitor of *Lmj*IPCS *in cellulo*. Further to this, clemastine **41** exhibited selectivity for wild type *L. major* promastigotes over sphingolipid-free mutant parasites, in which the IPC synthase enzyme is redundant. These findings support the hypothesis that clemastine **41** impedes *Leishmania major* growth *via* inhibition of IPCS.

Further work is in progress, towards gauging the effectiveness of clemastine **41** against other *Leishmania* species and progression to an *in vivo* cutaneous leishmaniasis model. This data will measure the potential of clemastine **41** as a therapy for *Leishmania* infection.

3. Clemastine Analogue Synthesis

This chapter focusses on an exploration of strategies with a view to synthesising a small library of analogues based on clemastine **41**. The synthesis and *in vitro* testing of nor-clemastine analogues (lacking one or two methyl groups), to assess the tractability of future synthetic efforts, will first be discussed. Then, a study into a number of connection strategies for library synthesis will be presented.

3.1 Review of the NINDS library set

Clemastine **41** was highlighted as the most promising *Lmj*IPCS inhibitor from the NINDS library screening campaign. In order to understand the interaction of clemastine **41** with the IPC synthase enzyme, a structure-activity study was necessary. As the *Leishmania* IPCS is a membrane-bound protein, hindering protein crystallography and spectroscopy efforts, SAR data would be gained through the screening of synthetic clemastine analogues.

A further analysis of the 1040 compounds within the NINDS set was conducted in order to draw any primary SAR data that could be built upon. A large number of *Lmj*IPCS inhibitors were found to be chemically similar to clemastine **41** (*i.e*. possess an aryl-rich moiety connected, through a flexible linker, to a nitrogen containing residue). However, these compounds displayed a lack of variation within the aryl and amino moieties. Furthermore, sporadic results of some compounds hampered attempts to correlate structure and activity, perhaps reflecting a presence of degraded material. Facing a dearth of concrete SAR data, it was decided to take an incremental approach to developing clemastine analogues. Only one half of the molecule would be altered at a time, giving rise to two separate series (Figure 3–1).

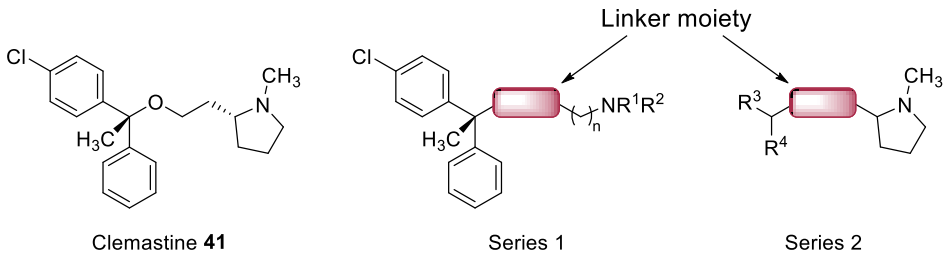


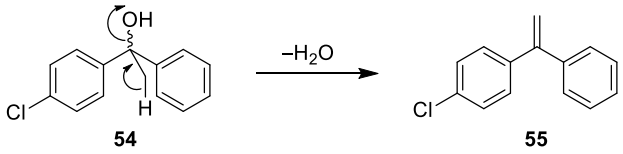
Figure 3–1: Strategy for clemastine analogue synthesis

A suitable linker would be required to allow for rapid access to a varied array of potential inhibitors. It was deemed appropriate to vary the linker initially as this represented the least functionalised part of clemastine **41** and hence it was hypothesised that changes to this moiety

would have the lowest effect on biochemical and antileishmanial activity. Using this strategy, a pharmacophore may be elucidated in a straightforward manner, without reliance on structural biology elements that are unavailable for the lipid-bound IPCS.

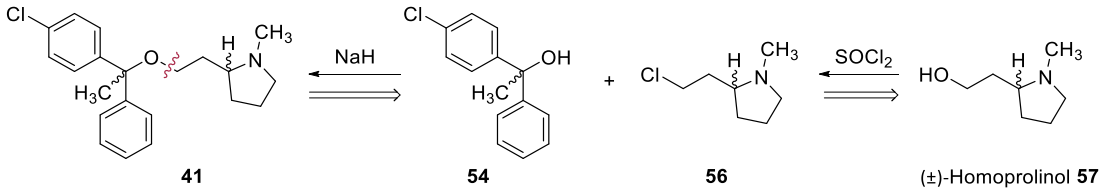
3.2 Synthesis of nor-clemastine analogues

Before an exploration of possible connection strategies could be made, research into the synthetic tractability of clemastine analogues was required. The necessity of optically pure material was a primary interest, followed by the requirement for a quaternary benzhydryl carbon. This latter concern was highlighted by studies within the group that demonstrated tertiary alcohol **54** was unstable towards formation of phenyl styrene **55** (Scheme 3–1). Additionally, the necessity of *N*-methylated pyrrolidine for IPCS inhibition was also probed. If activity against *Lmj*IPCS was unaffected by the presence of 1*H*-pyrrolidine, then future synthetic routes could utilise a protected nitrogen strategy, improving tractability.



Scheme 3–1: Instability of alcohol 54

Reported syntheses of clemastine **41** focussed on ether formation, *via* displacement of chloride **56** with deprotonated benzhydrylamine **54**, as the convergent step (Scheme 3–2).[220–222] Access to chloride **56** was achieved by activation of the precursor homoprolinol **57** using thionyl chloride. This synthetic route was employed for the construction of the desired nor-clemastine analogues.

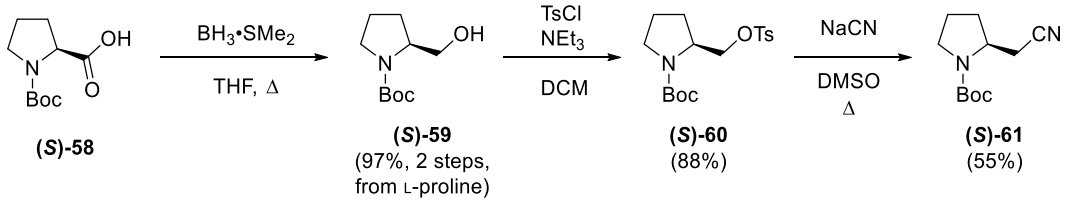


Scheme 3–2: Literature precedent for the synthesis of clemastine 41

3.2.1 Routes to homoprolinol

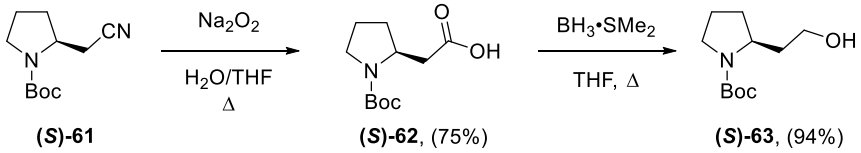
Initial efforts began with the attempted synthesis of homoprolinol **57**. A first route was explored involving straightforward homologation of proline **(*S*)-58** *via* nitrile **(*S*)-61** (Scheme 3–

3 and Scheme 3–4). The carbamate protected proline **(*S*)-58** was subjected to reduction by borane, furnishing prolinol **(*S*)-59** in good yield. The prepared alcohol **(*S*)-59** was found to have an optical rotation of $[\alpha]^{21}_{D} = -29.5°$(c = 1.00 g/100 mL, $CHCl_3$), comparable to the reported literature value of $[\alpha]^{25}_{D} = -39.8°$ (c = 1.20 g/100 mL, $CHCl_3$).[223] However, the enantiopurity of prolinol **(*S*)-59** was not experimentally verified *via* chromatography using a chiral column. Activation of the hydroxyl group by tosyl chloride provided the unstable tosylate **(*S*)-60** which was immediately displaced by a cyanide anion to access the desired nitrile **(*S*)-61** in modest yield (47%, 4 steps).[224] Synthesis of nitrile **(*S*)-61** was confirmed by the presence of a characteristic IR stretch at 2242 cm^{-1}. The chirality of nitrile **(*S*)-61** was inferred from the precursor alcohol **(*S*)-59**, although enantiopurity was not determined through methods involving optical rotation, chiral HPLC or NMR measurements.



Scheme 3–3: Synthesis of nitrile (*S*)-61 from L-proline

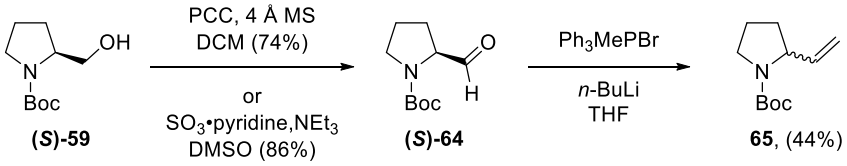
Next, a protocol reported by Vaughn *et al.* effected mild hydrolysis of nitrile **(*S*)-61** to furnish homoproline **(*S*)-62**,[225] corroborated by replacement of the characteristic nitrile stretch, in the IR spectrum, with that for the C=O bond at 1719 cm^{-1} (Scheme 3–4). Subsequent reduction using BH_3 afforded homoprolinol **(*S*)-63**, which displayed an optical rotation value of $[\alpha]^{28}_{D} = -28.3°$ (c = 1.00 g/100 mL, $CHCl_3$). The obtained value was found to vary from the literature value ($[\alpha]^{25}_{D}$ (c = 1.43 g/100 mL, $CHCl_3$) −10.7°),[223] which may be indicative of contamination or complex concentration-dependent dimer interactions.[226] A further investigation into the enantiopurity or the dependence of concentration of alcohol **(*S*)-63** on optical rotation was not performed.



Scheme 3–4: Synthesis of (*S*)-homoprolinol (*S*)-63

With an overall yield of 33% over 6 steps, encumbered particularly by the cyanide displacement step, a shorter, higher yielding route was desired. Attention turned towards a

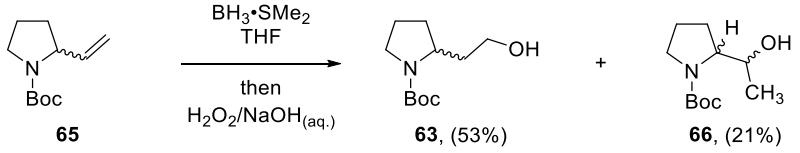
route utilising Wittig homologation with subsequent hydroboration (Scheme 3–5 and Scheme 3–6).



Scheme 3–5: Synthesis of olefin 65

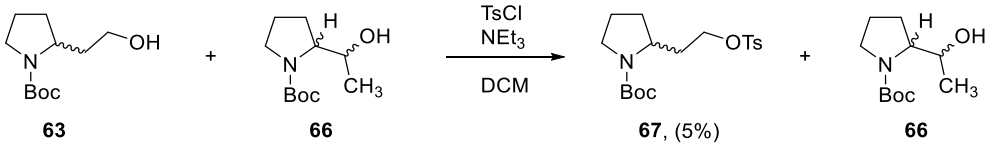
A brief investigation into the oxidation of prolinol **(*S*)-59** revealed Parikh-Doering oxidation, with SO_3·pyridine, to be more amenable to gram-scale reactions than the Jones variant with pyridinium chlorochromate.[227,228] Prolinal **(*S*)-64** was isolated in good yield, with the presence of the aldehydic proton verified by ^{1}H NMR ($\delta_H \sim 9.50$ ppm). Successive Wittig reaction with Ph_3MePBr proved to be lower yielding, proceeding with racemisation of the stereocentre, confirmed by optical rotation measurements of the olefin product **65**.[229] ^{1}H NMR analysis of the product revealed loss of the aldehydic proton, and appearance of olefinic signals at δ_H = 6.00 – 5.00 ppm corresponding to the isolation of alkene **65**.

Prolinal **(*S*)-64** was found to be unstable towards degradation over time, hindering further characterisation through measurement of its optical rotation. However, it was inferred that racemisation did not occur during the oxidation of prolinol **(*S*)-59** as the resultant aldehyde **(*S*)-64** was carried forth in other syntheses, with observed retention of stereochemistry (see Scheme 3–21, page 52). With olefin **65** in hand, it was then subjected to standard hydroboration using BH_3, followed by oxidation with hydrogen peroxide (Scheme 3–6).[223]



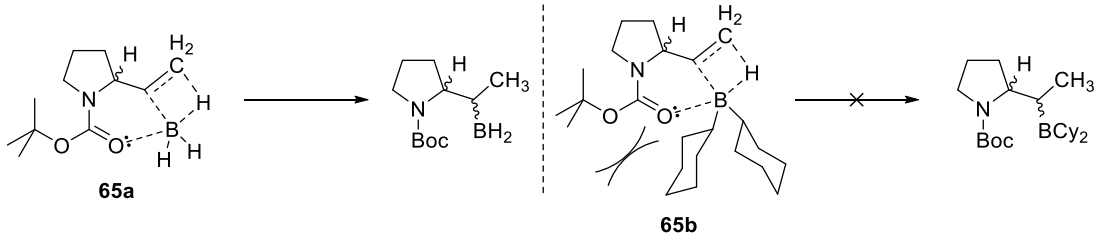
Scheme 3–6: Hydroboration of olefin 65 with BH_3

Surprisingly, the regioselectivity of this reaction proved to be poor, resulting in the production of isomers **63** and **66**, which could not be separated by flash chromatography on silica. However, secondary alcohol **66** was isolated successfully when the mixture was exposed to tosylation conditions (*vide supra*), which only converted homoprolinol **63** (Scheme 3–7).



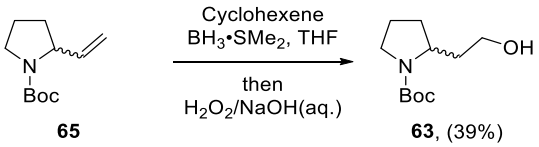
Scheme 3–7: Tosylation of alcohol mixture 63 and 66

The poor regioselectivity in the hydroboration step was postulated to occur due to co-ordination of the carbamoyl group of alkene **65** to borane, resulting in a lowering of the energy of transition state **65a** (Scheme 3–8). Addressing this issue by using a more hindered dialkylborane reagent, such as Cy_2BH, was assumed to negate this reactivity due to steric clashes preventing coordination.



Scheme 3–8: Hypothesis for poor the regioselectivity of BH_3 hydroboration of alkene 65

A modified hydroboration protocol was then carried out, with initial formation of dicyclohexylborane and successive exposure to alkene **65** (Scheme 3–9).[230] Subsequent oxidation furnished the desired alcohol **63** exclusively.



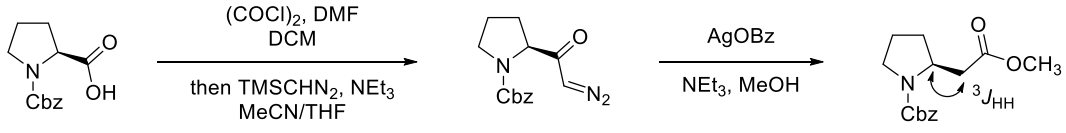
Scheme 3–9: Anti-Markovnikov addition of Cy_2BH to alkene 65 with following oxidation

Ultimately, although this procedure was successful, the overall yield was poor (14%, 5 steps). This, combined with the loss of enantiopurity rendered this synthetic sequence as unsuitable for further study.

A synthesis involving homologation *via* Arndt-Eistert chemistry, as previously demonstrated by Clayden's group, was utilised in which *N*-Z-proline **(*S*)-68** was initially converted to the diazoketone **(*S*)-69**, before Wolff rearrangement furnished the homologated ester **(*S*)-70** (Scheme 3–10).[222] This rearrangement appeared to operate with retention of stereochemistry, as indicated by optical rotation measurements, with ester **(*S*)-70** exhibiting a

$[\alpha]_D^{28}$ value of −30.2° (c = 1.00 g/100 mL, $CHCl_3$) (lit.:[231] $[\alpha]^{25}_D$ (c = 1.33 g/100 mL, $CHCl_3$) −36.6°). However, this finding was not corroborated experimentally by chiral HPLC or NMR methods, so the enantiomeric excess of compounds **(*S*)-68**, **(*S*)-69** and **(*S*)-70** could not be calculated.

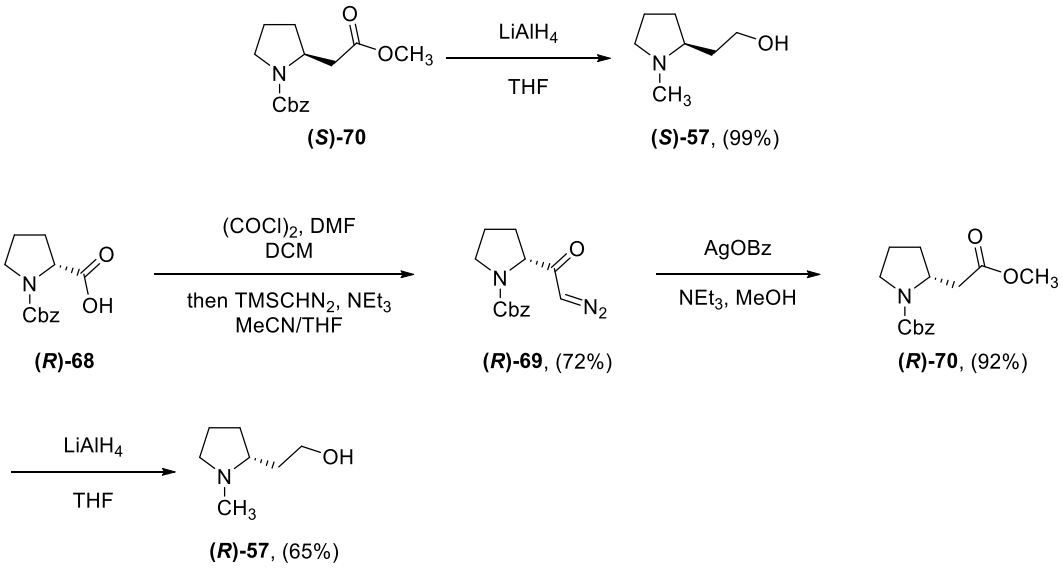
Successful rearrangement to form diazoketone **(*S*)-70** was confirmed through COSY 2D NMR experiment (shown below), which revealed mutual coupling between the ^1H chemical shifts at δ_H = 4.23 ppm (pyrrolidinyl CH) and δ_H = 3.01 – 2.34 ppm (α-carbonyl CH_2).



(*S*)-68 (*S*)-69, (76%) (*S*)-70, (82%)

Scheme 3–10: Arndt-Eistert reaction of *N*-Z-proline (*S*)-68

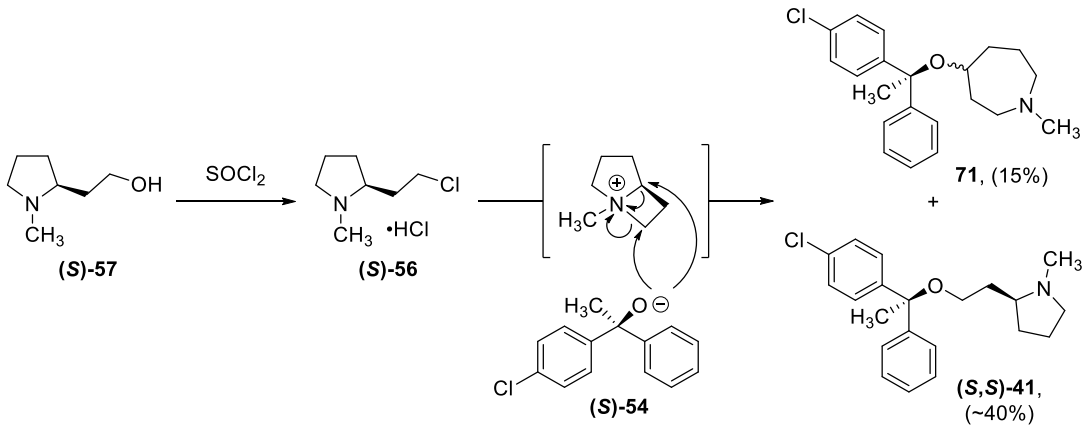
Global reduction of the β-amino acid ester **(*S*)-70** gave the desired (*S*)-*N*-methyl homoprolinol **(*S*)-57** in excellent yield, with loss of the carbonyl groups verified by ^{13}C NMR (Scheme 3–11). This route was then repeated with the unnatural (*R*)-proline **(*R*)-68** to rapidly access the desired homoprolinol **(*R*)-57.** This route proved to be the most efficient, providing yields of 62% and 43% across 4 steps for respective alcohols **(*S*)-57** and **(*R*)-57**.



Scheme 3–11: Reduction of ester (*S*)-70 and homologation sequence with the (*R*)-enantiomer

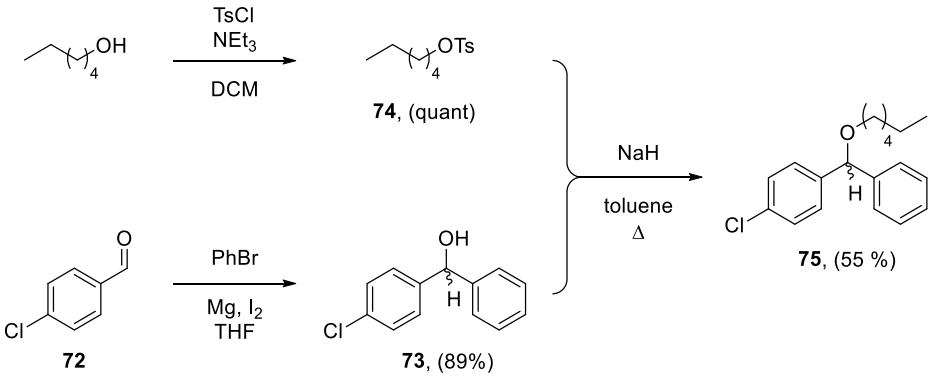
3.2.2 Etherification attempts

In the reported synthesis of (*S*,*S*)-clemastine **(*S*,*S*)-41**, Fournier *et al.* activated homoprolinol **(*S*)-57** to chloride **(*S*)-56**, prior to displacement with deprotonated benzhydrol **(*S*)-54** (Scheme 3–12).[222] However, anchimeric assistance by the amine N gave rise to azepane **71** as a by-product.



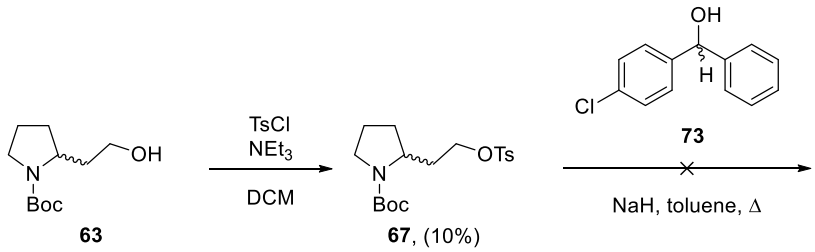
Scheme 3–12: Synthesis of (*S*,*S*)-clemastine (*S*,*S*)-41 by Fournier *et al.*[222]

It was hypothesised that activation of the Boc-protected homoprolinol **63** may circumvent this undesired reactivity by modulating nucleophilicity of the nitrogen atom. A test reaction was performed to assess whether tosylation was a viable activation strategy for displacement by benzhydrol **73** (Scheme 3–13). Facile reaction of benzaldehyde **72** and phenyl Grignard reagent furnished the benzhydrol **73**, which was reacted with tosylated hexanol **74**. Ether **75** was obtained in agreeable yield after chromatography on silica, confirmed by lack of an IR-active O–H stretch.



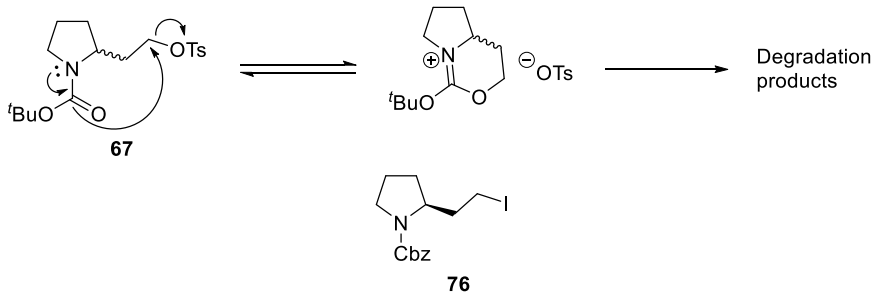
Scheme 3–13: Test displacement reaction with deprotonated benzhydrol 73

Next, a repeated tosylation attempt with *N*-Boc homoprolinol **63** resulted in a poor yield of tosylate **67**, with no starting material recovered *via* column chromatography (Scheme 3–14, *c.f.* Scheme 3–7). Furthermore, attempts to repeat this reaction proved capricious, pointing to an inherent instability of the activated alcohol **67**. This was confirmed as the compound degraded before full characterisation could be made. A final attempt to prepare the putative tosylate **67** immediately before use also proved to be fruitless and this route was abandoned.



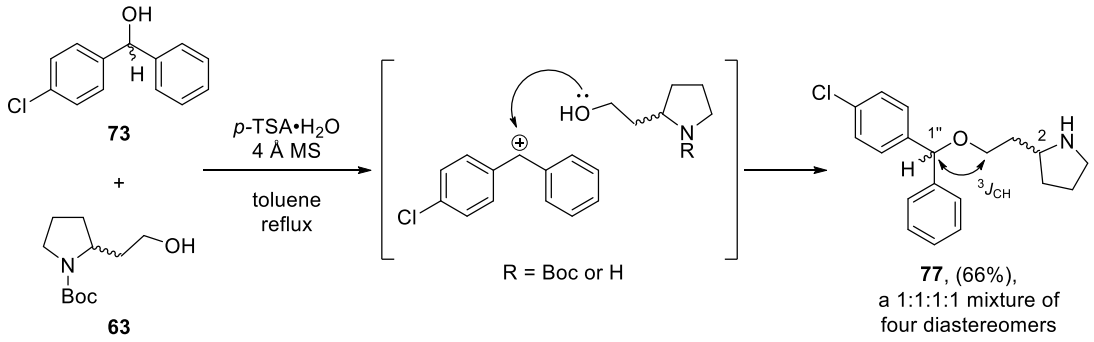
Scheme 3–14: Tosylation of homoprolinol 63 and attempted displacement reaction

The reason for the instability of tosylate **67** is unknown, but may occur *via* neighbouring group participation of the carbamate group (Scheme 3–15). Nevertheless, it was decided not to attempt alternative methods of activation, based on the observation by Clayden's group that the stable iodide analogue **76** was unreactive.[222]



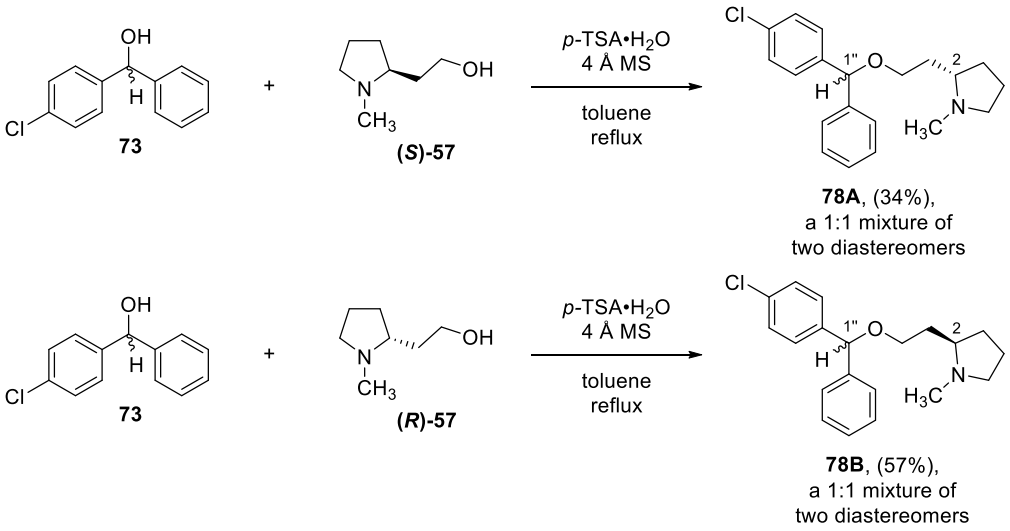
Scheme 3–15: Postulated instability of tosylate 67

It has been previously shown that benzhydrols can undergo acid-catalysed ether formation.[232,233] With this in mind, it was envisaged that *N*-Boc homoprolinol **63** would undergo simultaneous etherification and deprotection to afford the desired nor-clemastine analogue **77** (Scheme 3–16). Gratifyingly, heating alcohols **63** and **73** under reflux with 1.1 eq. of *p*-toluenesulfonic acid in the presence of 4 Å molecular sieves (4 Å MS) furnished ether **77**, as a mixture of the four diastereomers (2*R*,1''*R*), (2*R*, 1''*S*), (2*S*,1''*R*) and (2*S*,1''*S*), in reasonable yield. Preferential C-O bond formation over C-N bond formation was confirmed by the HMBC correlation depicted below.



Scheme 3–16: Condensation of alcohols 63 and 73

This methodology was subsequently applied to methylated alcohols **(*S*)-57** and **(*R*)-57** to provide the respective clemastine analogues **78A** and **78B**, as mixtures of diastereomers (Scheme 3–17). Due to the postulated reaction pathway, the formation of these diastereomeric mixtures was viewed as unavoidable. Separation of the respective diastereomers of compounds **78A** and **78B** *via* chromatography was found to be unfeasible, as no separation was observed by both TLC and HPLC.



Scheme 3–17: Synthesis of clemastine analogues 78A and 78B

It must also be noted that the enantiomeric excess of homoprolinol compounds **(*S*)-57** and **(*R*)-57** was not measured, and their chirality was inferred from the optical rotation measurements of the respective ester precursors **(*S*)-70** and **(*R*)-70**. The implications of this are that there may be trace amounts of the undesired diastereomers (2*R*,1''*R*) and (2*R*,1''*S*), in mixture **78A**, and (2*S*,1''*R*) and (2*S*,1''*S*), in mixture **78B**.

3.2.3 *In vitro* activity of nor-clemastine analogues

With analogues **77**, **78A** and **78B** in hand, attention then turned towards measuring their activity against *Leishmania major* IPC synthase and promastigotes. A preliminary dose-response investigation into inhibition of *Lmj*IPCS revealed all analogues to have micromolar activity (Figure 3–2).

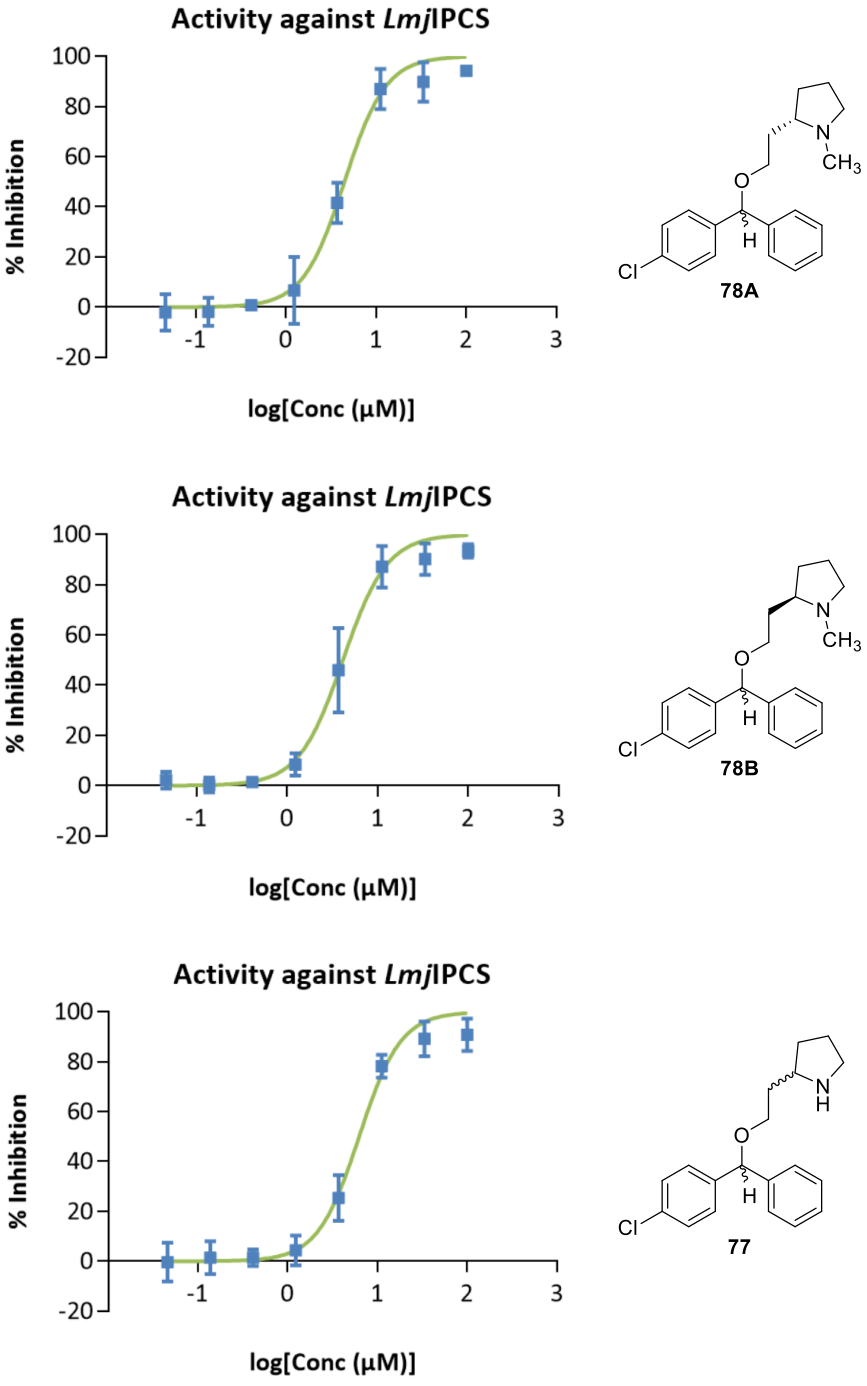


Figure 3–2: Biochemical activity of analogues 78A, 78B and 77. Error bars portray 95% confidence interval.

Compared to clemastine **41** (IC_{50} = 2.87 µM, 95% CI: 2.25 – 3.66 µM), analogues **78A** and **78B** (lacking one methyl group) exhibited respective IC_{50} values of 4.45 µM (95% CI: 4.01 – 4.94 µM) and 4.13 µM (95% CI: 3.72 – 4.59 µM). The relative lower activity of these compounds may be explained by the absence of a benzhydryl methyl group, pointing to potential interactions between the *Lmj*IPCS enzyme and the hydrophobic region of the molecule. From the similar IC_{50} values calculated for the diastereomeric mixtures **78A** and **78B**, it is not possible to assert whether pyrrolidine stereochemistry has an effect on compound potency. To assess this, it would be necessary to isolate and screen the four separate diastereomers within these mixtures. Specifically, testing the (2*R*,1''*R*) diastereomer of compound mixture **78B**, the closest analogue to (*R*,*R*)-clemastine **41**, would allow for a direct comparison, giving a definitive answer as to the effect, if any, of benzhydryl *C*-methylation on activity.

The potency of analogue **77** (lacking two methyl groups) was found to be even lower (IC_{50} = 6.26 µM, 95% CI: 5.60 – 7.00 µM), suggesting that presence of the tertiary amine group is important for IPCS inhibition. The drop in potency may be due to lowered amine basicity and nucleophilicity.

The effect of methylation on antileishmanial activity was then probed (Figure 3–3). Interestingly, the mixture **78B** exhibited sub-micromolar activity (ED_{50} = 0.78 µM, 95% CI: 0.63 – 0.96 µM), whereas the complementary analogue **78A** was ~2-fold less effective (ED_{50} = 1.69 µM, 95% CI: 1.23 – 2.32 µM). This indicates that one, or both, of the (2*R*,1''*S*) and (2*R*,1''*R*) diastereomers within mixture **78B** has a higher antileishmanial effect than the (2*S*,1''*R*) and (2*S*,1''*S*) isomers of mixture **78A**. However, this would need to be experimentally verified through the testing and analysis of all four separated isomers, which may in turn uncover important links between *Lmj*IPCS inhibition and antiparasitic activity. It is pertinent to note that the observed difference in potency may be due to improved cell penetration and transport to *Lmj*IPCS, or lower susceptibility to removal *via* metabolism or efflux. Additionally, the potential for interaction with other target enzymes cannot be ruled out as a potential contributing factor.

Desmethyl analogue **77** was found to have significantly lower activity against *Leishmania major* (ED_{50} = 4.10 µM, 95% CI: 3.35 – 5.01 µM), in agreement with the observed impact on IPCS inhibition. As with clemastine **41**, analogues **78A**, **78B** and **77** all exhibited increased potency against *L. major* promastigotes over the IPC synthase (*c.f.* Figure 2–2). As

discussed in Chapter 2, this may be due to pleiotropic effects, increased relative concentration around the Golgi apparatus, or metabolism to compounds with increased potency.

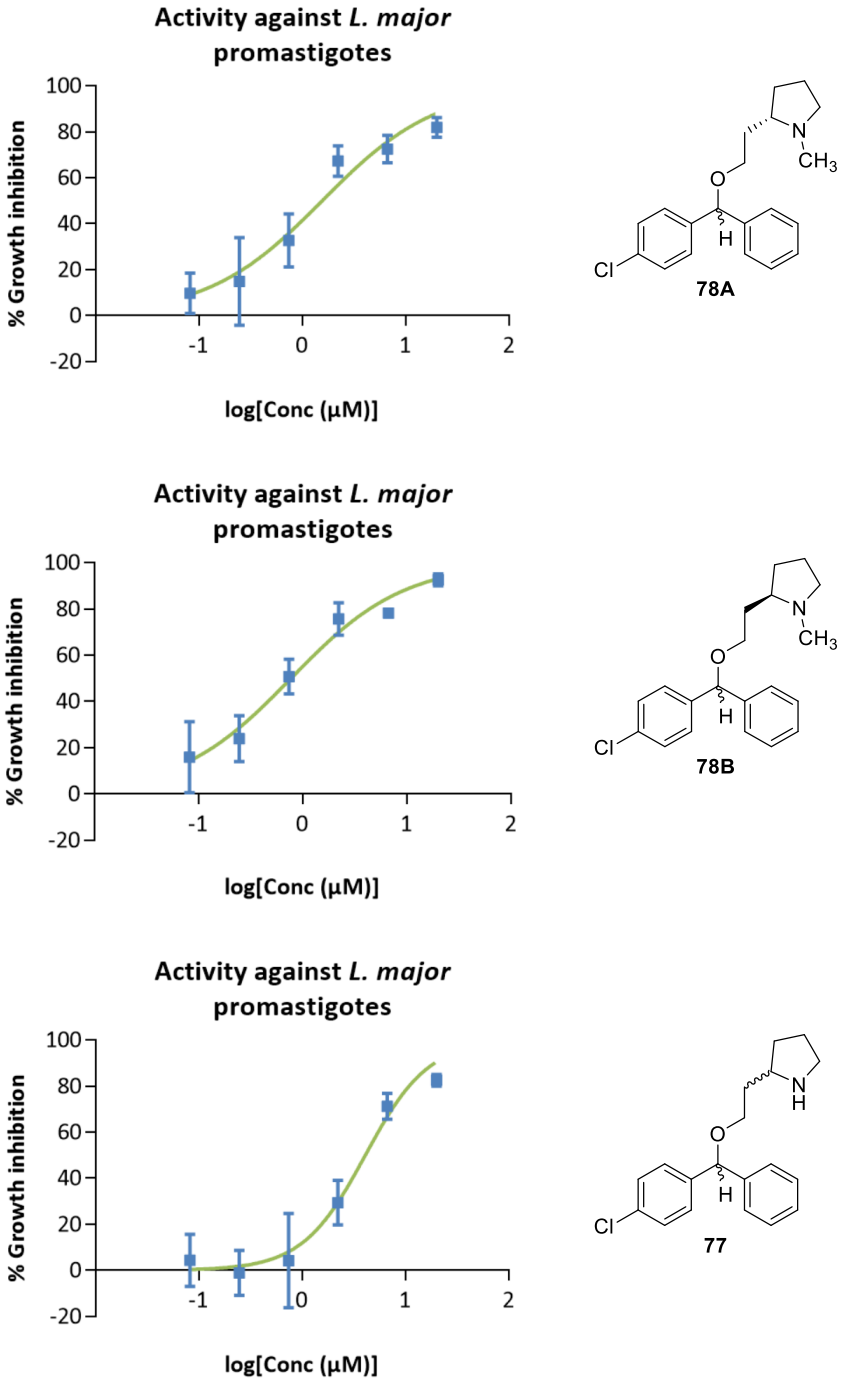


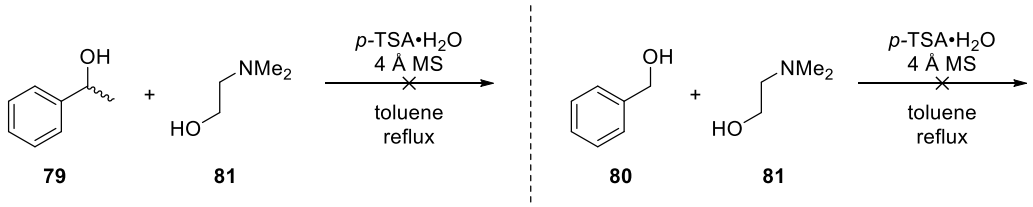
Figure 3–3: Leishmanicidal activity of analogues 78A, 78B and 77. Error bars portray 95% confidence interval.

In conclusion, a brief investigation into the synthetic tractability of clemastine **41** analogues revealed that a lack of methylation on the pyrrolidinyl nitrogen, or benzhydryl carbon, did not ablate inhibition of IPCS or parasite growth. More specifically, the presence of *N*-methylated

pyrrolidine appears to result in moderately improved activity against the *Lmj*IPCS enzyme and *Leishmania major* parasites. Furthermore, it seems that the stereochemistry of the 2-position of the pyrollidine group has an impact on potency against *L. major* promastigotes, although (*R*,*R*) stereochemistry, such as that of clemastine **41**, is not vital for activity. However, this observation, and any potential link to IPCS inhibition, requires further exploration through testing of the separate diastereomers comprising the mixtures **78A** and **78B**.

3.3 Possible routes for library synthesis

As described earlier (Figure 3–1), accessing a diverse library of clemastine analogues required a straightforward connection strategy. The condensation methodology utilised for the syntheses of nor-clemastine analogues **78A**, **78B** and **77** may be useful in this context, but was restricted by the requirement for benzhydryl alcohols as substrates. This was confirmed by the reaction below in which 1-phenyl ethanol **79** and benzyl alcohol **80** failed to react with amino alcohol **81** (Scheme 3–18).



Scheme 3–18: Alcohols 79, 80 and 81 do not react under acid-catalysed etherification conditions

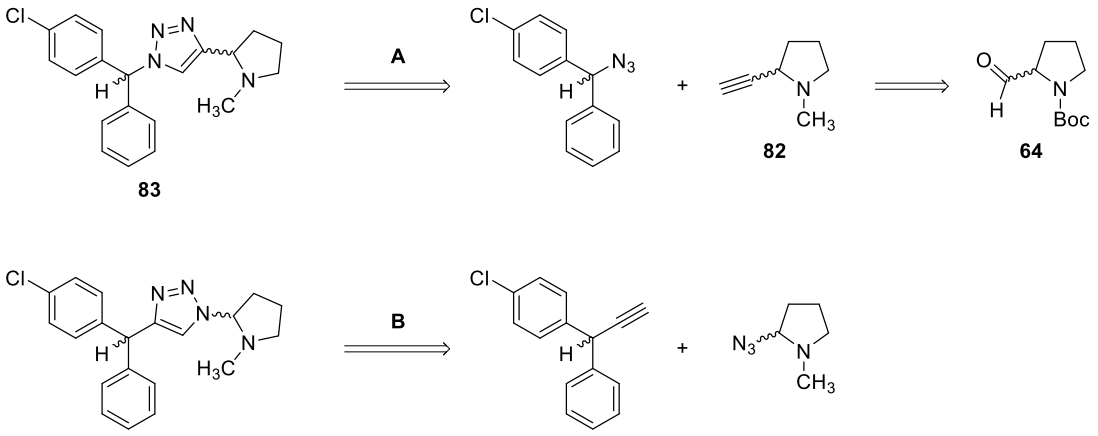
Therefore, a number of different strategies were explored with a view to increasing the substrate scope for analogue synthesis. Three commonly used tactics were employed in parallel, namely Cu(I)-catalysed 1,2,3-triazole synthesis, amide and ester bond formation, and alkene metathesis. The simultaneous *in vitro* testing of analogues synthesised using these methods would allow for a direct comparison to be made, highlighting the best strategy for further synthetic efforts.

3.3.1 Triazole analogue synthesis

1,2,3-Triazoles are popular in medicinal chemistry due to their biocompatibility and stability under physiological conditions.[234] Furthermore, their creation *via* 'click chemistry' is ideal due to the high efficiency and functional group tolerance that characterise this transformation.

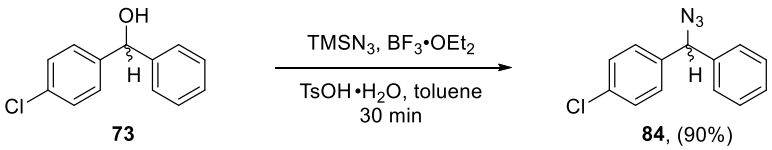
Thus, an exploration into the synthesis of clemastine analogues containing a 1,2,3-triazole linker was carried out.

It was apparent that two different routes could be followed to furnish a triazole-linked clemastine analogue (Scheme 3–19). It was viewed that the pyrrolidine fragment **82** could be accessed from the previously prepared prolinal **64**, potentially expediting the synthesis of triazole **83**. Therefore, route A was preferentially chosen for the synthesis of a triazole-linked analogue of clemastine.



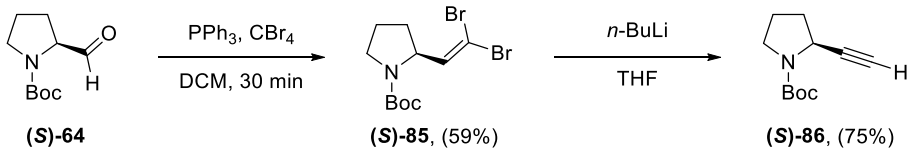
Scheme 3–19: Synthetic routes to triazole-linked clemastine analogues

Synthesis of the azide fragment proved facile, with the conversion of benzhydrol **73** to azide **84** occurring in high yield on reaction with $TMSN_3$ and $BF_3{\cdot}OEt_2$ (Scheme 3–20).[235] The successful introduction of the azide functionality was confirmed by the observation of a characteristic stretch at 2095 cm^{-1} in the IR spectrum.



Scheme 3–20: Conversion of alcohol 73 to azide 84

Accessing the corresponding alkyne fragment **(*S*)-86** from prolinal **(*S*)-64** was also straightforward, through utilisation of Corey-Fuchs methodology (Scheme 3–21).[228]



Scheme 3–21: Corey-Fuchs reaction, providing alkyne (*S*)-86

Dibromoalkene **(*S*)-85** was synthesised in modest yield, confirmed by mass spectrum revealing a characteristic dibromo isotope pattern (*m/z* = 354 [M($^{79}Br_2$)H^+], 356 [M($^{79}Br^{81}Br$)H^+], 358 [M($^{81}Br_2$)H^+]), which was then converted to alkyne **(*S*)-86**. Analysis of the ^{1}H NMR spectrum revealed disappearance of the olefinic proton (δ_H = 6.38 – 6.25 ppm) and appearance of a signal at δ_H = 2.21 – 2.15 ppm, consistent with the presence of a terminal alkyne. The measured optical rotations of compounds **(*S*)-85**, $[\alpha]^{21}_D$ = +8.5° (c = 1.00 g/100 mL, $CHCl_3$) (lit.:[228] $[\alpha]^{26}_D$ = +24.0° (c = 0.89 g/100 mL, DCM)), and **(*S*)-86**, $[\alpha]^{27}_D$ = −49.5° (c = 1.00 g/100 mL, $CHCl_3$) (lit.:[236] $[\alpha]^{25}_D$ = −66.3° (c = 1.35 g/100 mL, $CHCl_3$)), indicated an excess of the desired *S*-enantiomer. However, further verification was not obtained through chiral HPLC or NMR methods, meaning the exact enantiomeric excess could not be calculated. As discussed earlier in this chapter, this carries the implication that a potentially unknown, albeit likely to be small, quantity of the opposite *R*-enantiomer may be present in subsequent synthetic steps.

With alkyne **(*S*)-86** and azide **84** in hand, their connection *via* Cu(I)-catalysed Huisgen cycloaddition was investigated.[237] Initially, the reaction of these fragments at RT was explored, resulting in very little conversion of the starting materials **84** and **(*S*)-86**, as observed by TLC (Table 3–1 entry 1). Slight elevation of the temperature to 40 °C similarly had no effect. It has been reported by Bouillon *et al*. that microwave assisted cycloaddition occurs rapidly.[238] Following this procedure, alkyne **(*S*)-86** and azide **84** were combined in a 1.1 : 1 ratio respectively and heated at 60 °C for 20 min, yielding cycloadduct **87A** in 70% yield (Table 3–1, entry 2). Optimisation of these conditions was briefly explored, with 79% of the diastereomeric mixture **87A** being isolated following a longer reaction time of 30 min (Table 3–1, entry 3). Reaction of **(*S*)-86** and **84** in a 1 : 1 ratio resulted in a lower yield, even with extension of the heating time to 40 min (Table 3–1, entry 4).

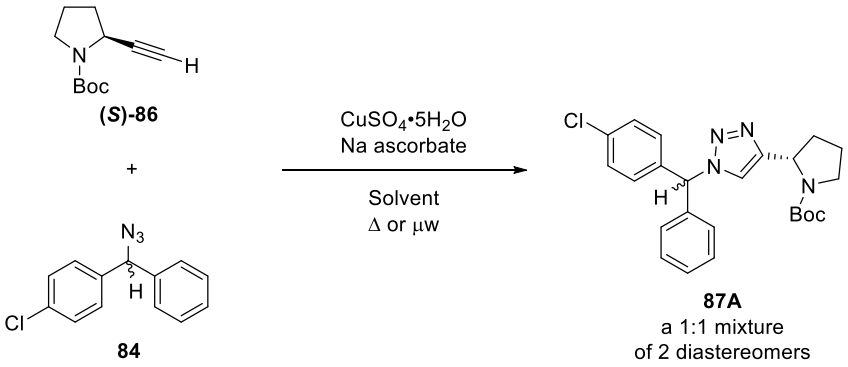


Table 3–1: Cu(I) catalysed 1,2,3-triazole formation

Entry	Ratio (*S*)-86 : 84	Eq. Cu(I)	Eq. Na ascorbate	Δ / μW	Solvent	Time	Yield 87A
1	1 : 1	0.02	0.1	Δ, 40 °C	^{t}BuOH/H_2O	2 days	–
2	1.1 : 1	0.4	2	μW, 60°C	MeOH/H_2O	20 min	70 %
3	1.1 : 1	0.4	2	μW, 60°C	MeOH/H_2O	30 min	79 %
4	1 : 1	0.4	2	μW, 60°C	MeOH/H_2O	40 min	65 %

It was hypothesised that the success of the microwave reaction over the thermal reaction (Table 3–1, entries 1 & 4), may be due to the relatively low equivalents of $CuSO_4$ and Na ascorbate employed in the former reaction. This, however, remains to be experimentally verified.

The absence of the characteristic IR azide stretch at 2095 cm^{-1} was noted in the purified product **87A**, as well as the significantly higher chemical shift of the benzhydryl proton (δ_H = 7.71 ppm), observed in the ^{1}H NMR spectrum. Triazole **87A** was also found to be rotameric, with variable temperature NMR experiments demonstrating coalescence of peaks at 90 °C in DMSO-d_6 (Figure 3–4).

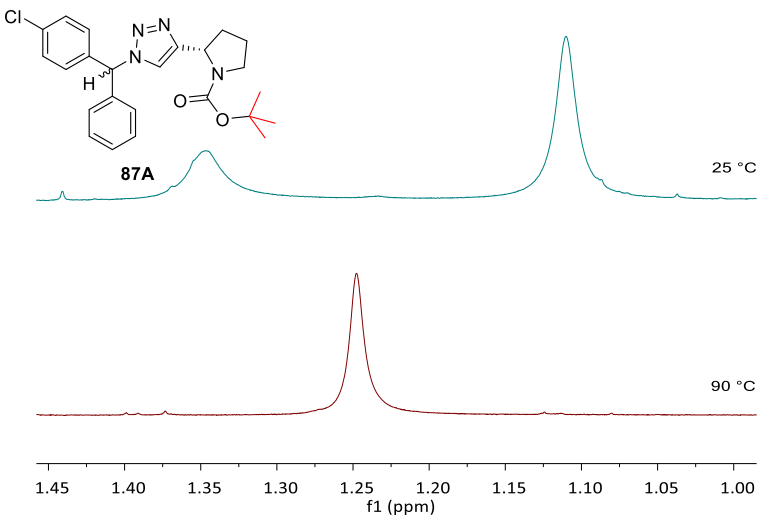
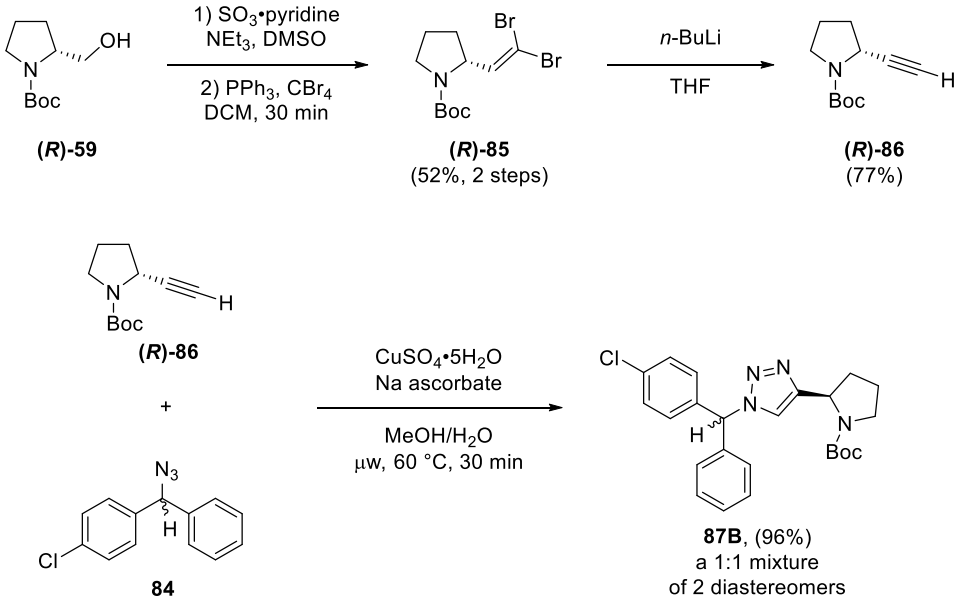


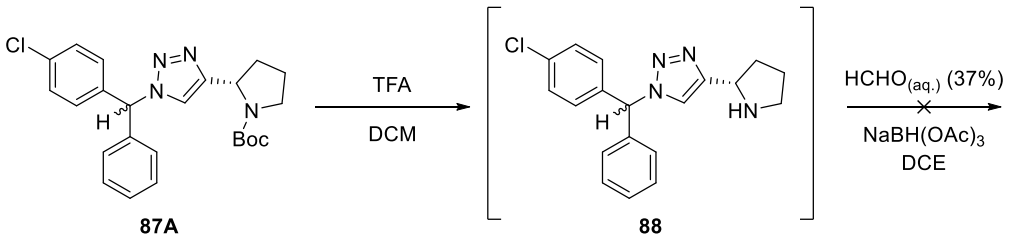
Figure 3–4: VT ^{1}H NMR experiment with triazole 87A (*tert*-butyl peaks shown)

With a viable synthetic route in hand, attention then turned towards its application to (*R*)-*N*-Boc prolinol **(*R*)-59** (Scheme 3–22). Alkyne **(*R*)-86** was subsequently prepared in 40% yield over 3 steps, with successive click reaction of fragments **84** and **(*R*)-86** furnishing triazole **87B**, as a mixture of diastereomers, in almost quantitative yield.



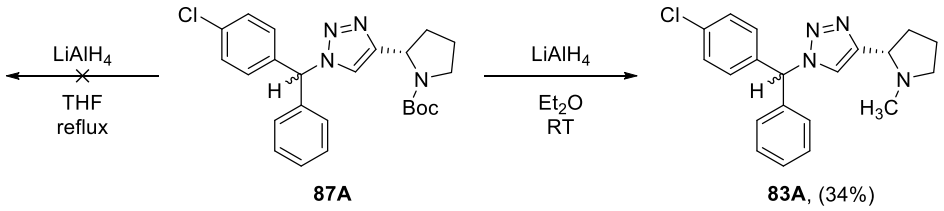
Scheme 3–22: Synthetic route to cycloadduct 87B

A number of strategies were employed in the attempted synthesis of *N*-methyl triazole **83** (Scheme 3–23 and Scheme 3–24). A primary effort involved acid mediated Boc deprotection of triazole **87A**, followed by treatment of the deprotected amine **88** with aq. formaldehyde and $NaBH(OAc)_3$, according to precedent by Abdel-Magid *et al.* (Scheme 3–23).[239] Unfortunately a complex mixture resulted, from which only 34% of unprotected amine **88** could be recovered.



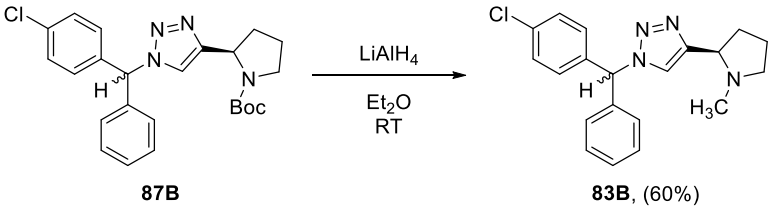
Scheme 3–23: Failed reductive amination attempt

Reduction of Boc-protected analogue **87A** was also investigated in parallel (Scheme 3–24). An initial attempt utilising $LiAlH_4$ in THF at reflux resulted in degradation of the starting material **87A** to an intractable mixture, as judged by TLC and ^{1}H NMR of the crude material.[240] A second trial reaction at ambient temperature, using Et_2O as solvent, resulted in clean conversion of starting material observed by TLC.[241] Following purification by chromatography on silica, the desired *N*-methyl analogue **83A** was obtained in 34% yield. Analysis of the ^{1}H NMR spectrum of this compound revealed absence of the *tert*-butyl shift of analogue **87A**, and appearance of a new singlet peak at δ_H = 2.26 ppm. Additionally, full resolution of peaks was observed in the ^{1}H NMR spectrum of triazole **83A**, contrasting with the observation of rotamers with analogue **87A** (*c.f.* Figure 3–4).



Scheme 3–24: Hydride reduction of *N*-Boc triazole 87A

Following this, $LiAlH_4$ reduction of analogue **87B** furnished the corresponding *N*-methyl triazole **83B** in acceptable yield (Scheme 3–25).



Scheme 3–25: Hydride reduction of *N*-Boc triazole 87B

In summary, triazole-linked analogues **83A** and **83B** were accessed after successful microwave-mediated Cu(I)-catalysed formal cycloaddition. This connection step proved to be

fast and high yielding and so could be used effectively for the synthesis of an array of clemastine analogues.

3.3.2 Amide and ester analogue synthesis

Acylation reactions, notably peptide bond formation, are a key tool within the medicinal chemist's repertoire.[242,243] Hence, routes to ester and amide linked clemastine analogues were explored. A brief analysis revealed that six fragments were required (Figure 3–5). For ease of purification it was decided to keep the pyrrolidinyl nitrogen protected throughout the synthetic route. Furthermore, to improve tractability of synthesis, *N*-methylation would not be pursued as a final step.

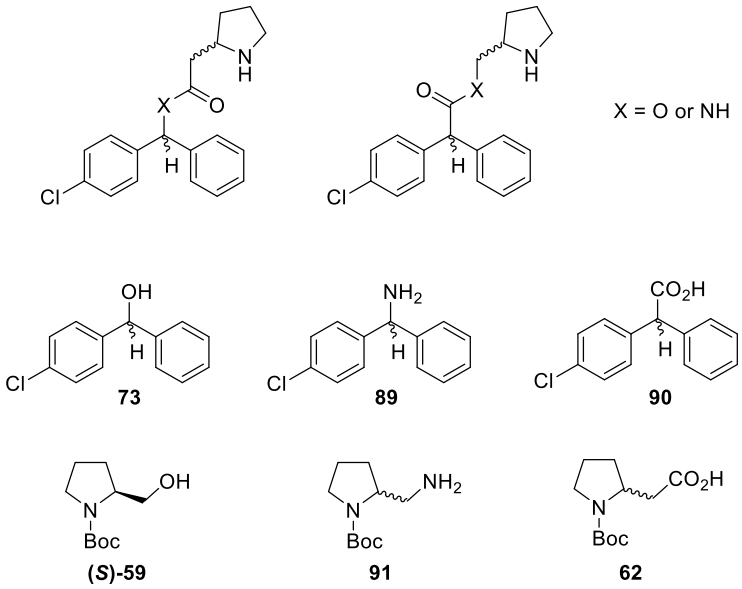
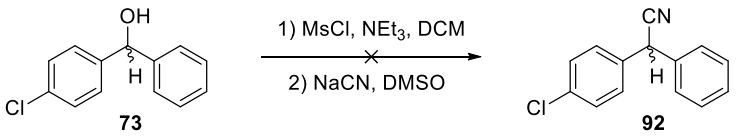


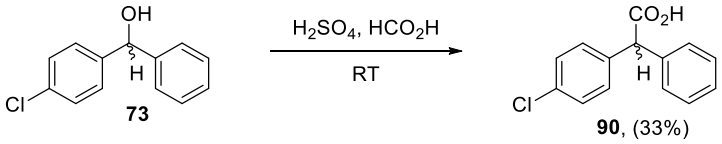
Figure 3–5: Precursors required for synthesis of acyl-linked clemastine analogues

Alcohols **(*S*)-59** and **73** were synthesised as previously described (Scheme 3–3 and Scheme 3–13), with the other fragments **62**, **89** and **91** purchased from commercial sources. However, acid **90** was not available and needed to be prepared. Initial approaches explored the homologation of benzhydrol **73** (Scheme 3–26), however, the nitrile **92** could not be isolated.



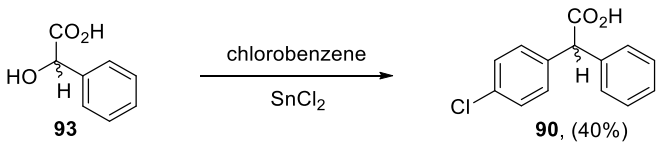
Scheme 3–26: Failed homologation attempt of benzhydrol 73

Next, a precedent was followed in which a benzhydryl cation was formed from alcohol **73** in strong acid, prior to trapping by carbon monoxide formed *in situ* (Scheme 3–27).[244] Whilst the presence of a carbonyl group was verified by an IR-active stretch at 1707 cm^{-1}, the desired acid **90** could only be isolated in low yield.



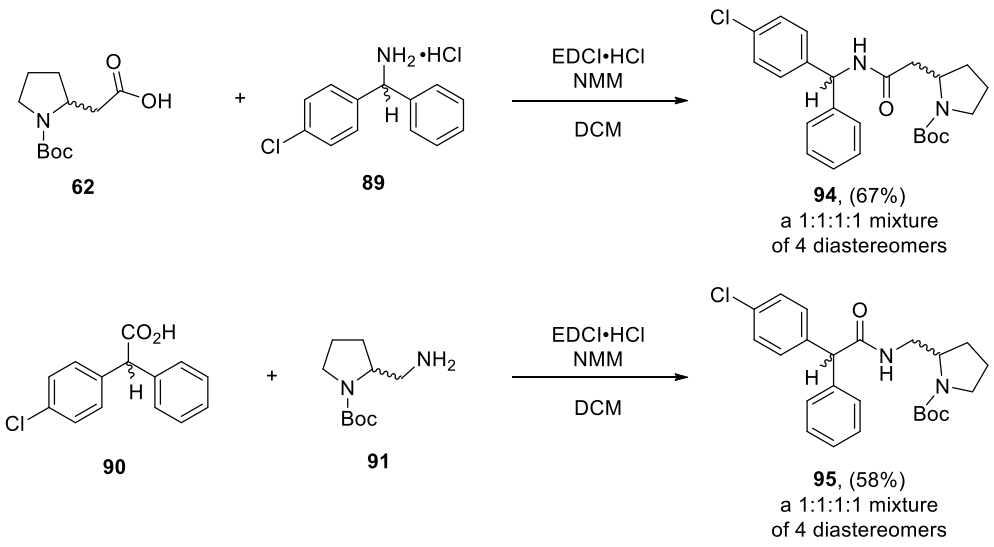
Scheme 3–27: Synthesis of acid 90

In an effort to access acid **90** in more acceptable yield, a protocol utilising Lewis-acid mediated Friedel-Crafts arylation of mandelic acid **93** was employed (Scheme 3–28).[245] Following re-crystallisation of the crude product from acetone and petrol, the acid **90** was provided in modest but acceptable yield (40%).



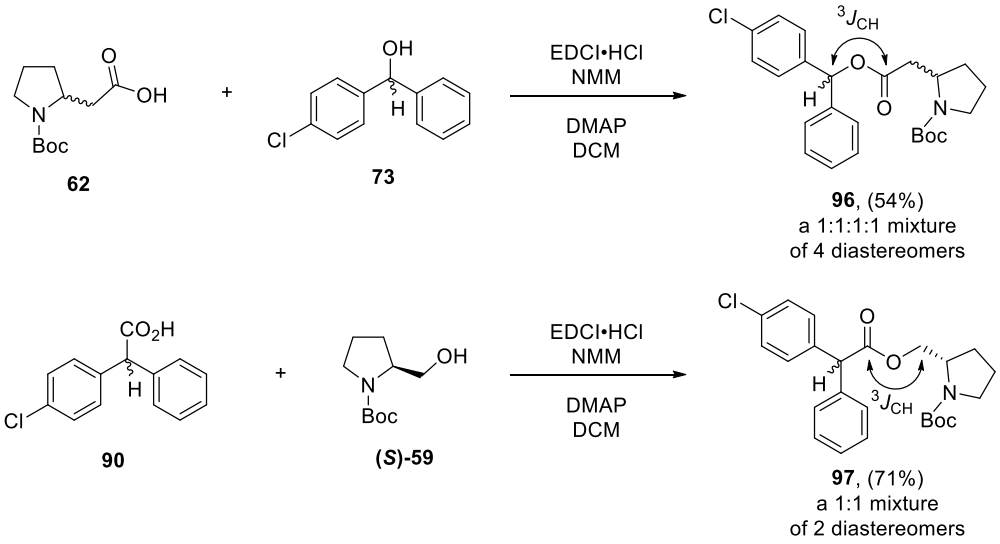
Scheme 3–28: Friedel-Crafts arylation of mandelic acid 93

With all the required fragments in hand, attention turned toward the synthesis of amide and ester linked clemastine analogues (Scheme 3–29 and Scheme 3–30). Using carbodiimide as an activating reagent, amides **94** and **95** were synthesised in reasonable yield (Scheme 3–29).[246] Successful peptide bond formation was confirmed by the presence of distinctive IR-active vibrations at *circa* 1650 cm^{-1} and 1540 cm^{-1}.



Scheme 3–29: Synthesis of amide analogues 94 and 95

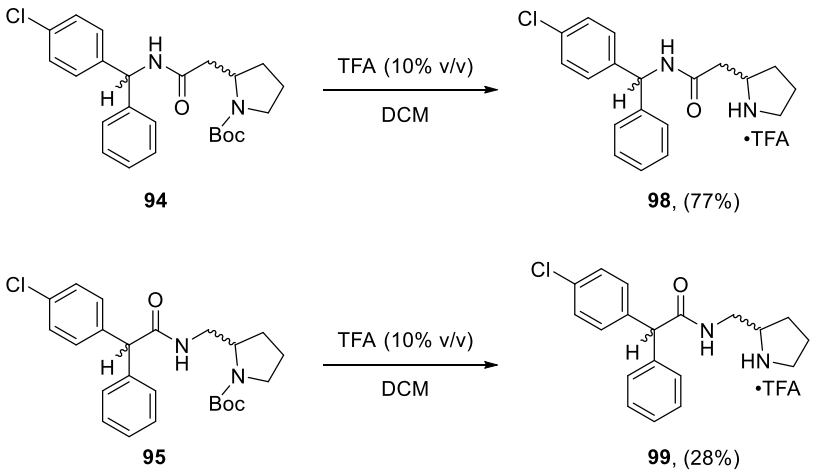
Ester bond formation was effected with the addition of EDCI and 1.1 eq. DMAP, furnishing analogues **96** and **97** in good yield (Scheme 3–30). The ^{1}H and ^{13}C NMR spectra of these esters were complicated by the presence of both diastereoisomers and rotamers. However, effective acylation was verified by $^{3}J_{CH}$ correlations (HMBC) as depicted in Scheme 3–30.



Scheme 3–30: Synthesis of ester analogues 96 and 97

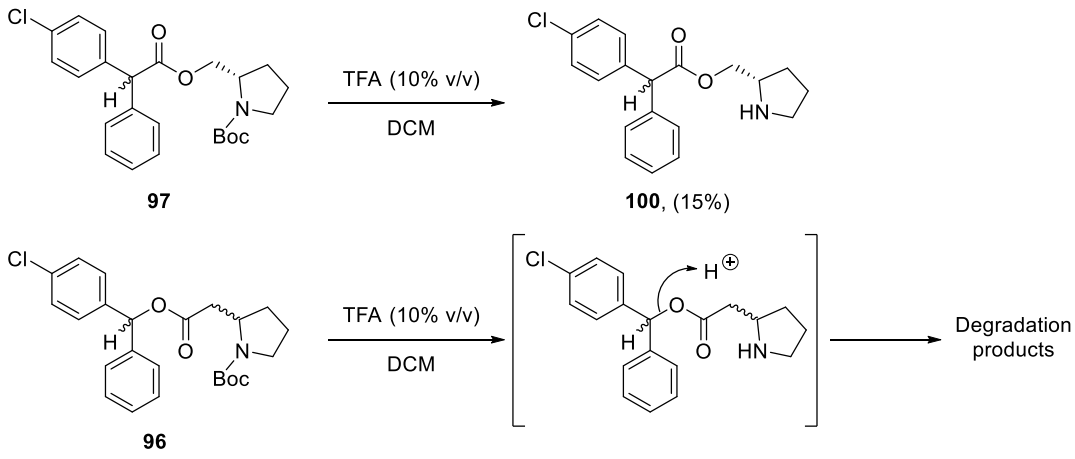
Acid mediated carbamate deprotection of amide **94** occurred cleanly, providing TFA salt **98** in good yield (Scheme 3–31). In contrast, amine **99**, derived from the amide **95**, was isolated in 28% yield after re-crystallisation of the crude material from EtOAc and ether. The reasons behind this disparity in yield remain unclear. Analysis of ^{13}C NMR spectra revealed

disappearance of the carbamate acyl carbon resonance at δ_C = 155 ppm, supporting effective deprotection.



Scheme 3–31: Acid mediated Boc cleavage of amides 94 and 95

Esters **96** and **97** were subjected to the same deprotection conditions (Scheme 3–32). Analogue **100** could not be cleanly isolated as either a TFA or *p*-TSA salt, and so the free amine was purified *via* preparative TLC. Unfortunately, the acidic conditions led to complete degradation of the protected ester **96**, presumably due to the propensity for benzhydryl cation formation.



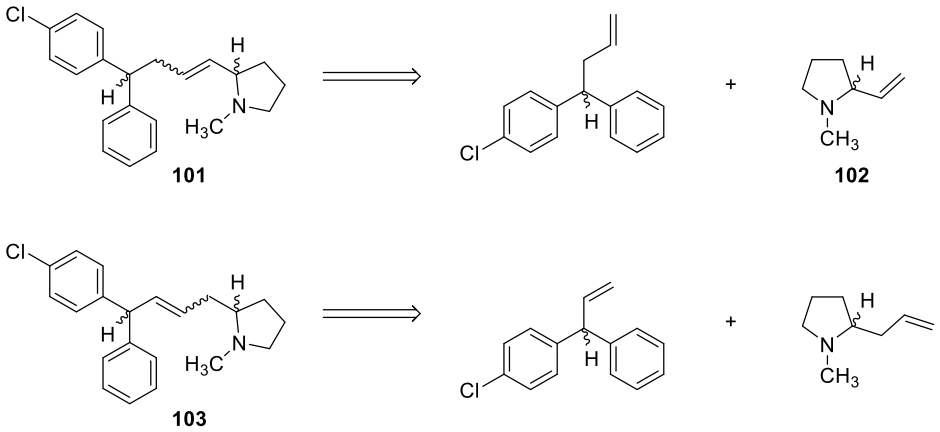
Scheme 3–32: Attempted carbamate deprotection of esters 96 and 97

To conclude, *N*-Boc clemastine analogues **94**, **95**, **96** and **97** were readily accessed *via* carbodiimide-mediated *N*- and *O*-acylation. However, subsequent deprotection attempts resulted in the decomposition of ester **96**, revealing the esterification route to have lower

functional group tolerance. Therefore, amide bond formation represented a more tractable synthetic strategy.

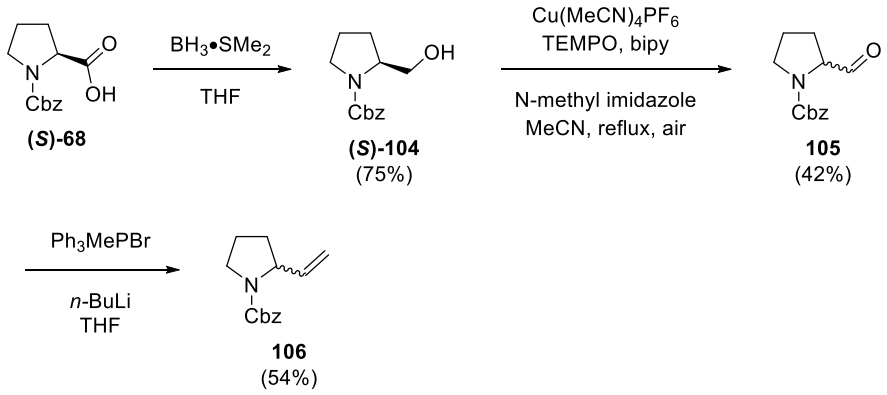
3.3.3 Olefin analogue synthesis

Olefin metathesis is another popular linking strategy, characterised by high efficiency and functional group tolerance.[247,248] A brief investigation into the tractability of this method was performed. Two routes to an alkene linked clemastine analogue were considered (Scheme 3–33). The route to analogue **101** was viewed as the most expedient, as vinyl pyrrolidine **102** was easily accessible (see Scheme 3–5 above).



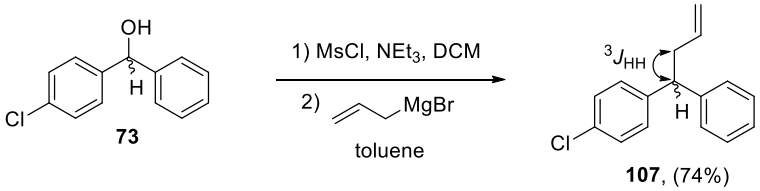
Scheme 3–33: Potential routes to olefin analogues 101 and 103

With this in mind, Cbz-protected proline **(*S*)-68** was chosen as a precursor (Scheme 3–34), as the carbamate could be subjected to reduction using $LiAlH_4$ to give an *N*-methylated product (see Scheme 3–11). Additionally, exposure to hydrogenation conditions would effect simultaneous deprotection and saturation of the alkene moiety, introducing flexibility. Aldehyde **105** was prepared from proline **(*S*)-68** in modest yield (32% over 2 steps), with the presence of the characteristic aldehydic proton observed by ^{1}H NMR (δ_H = 9.60 – 9.50 ppm).[249,250] Wittig olefination of prolinal **105** furnished the desired alkene **106**, as evidenced by resonances at δ_H = 5.82 – 5.71 ppm and 5.19 – 5.08 ppm in the ^{1}H NMR spectrum.[229]



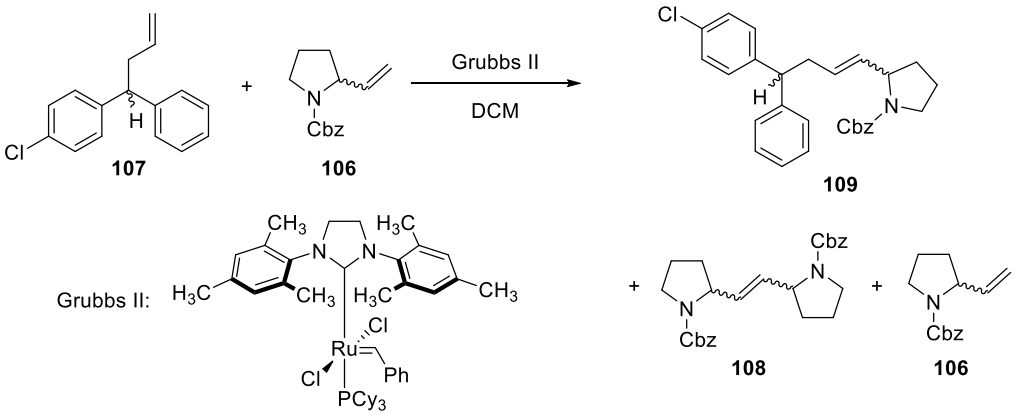
Scheme 3–34: Synthetic route to alkene 106 from (*S*)-*N*-Z-proline (*S*)-68

The complementary fragment **107** was accessed by activation of alcohol **73** with mesyl chloride, prior to displacement by allyl magnesium bromide (Scheme 3–35). Analysis of a 2D NMR COSY experiment confirmed mutual coupling between the signals at δ_H = 3.99 ppm and 2.84 – 2.74 ppm, corresponding to the hydrogens at the benzylic and allylic positions respectively.



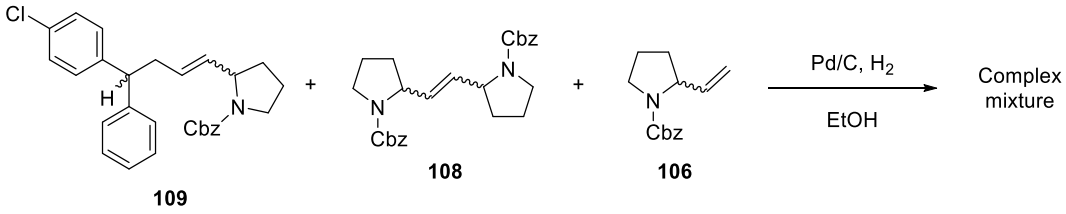
Scheme 3–35: Conversion of benzhydrol 73 to olefin 107

Application of cross metathesis conditions, using the Grubbs' generation II catalyst, led to the generation of a number of products, as revealed by TLC and LCMS analysis of the crude material (Scheme 3–36).[251] In particular, the pyrrolidine dimer **108** (*m/z* = 435 [MH^+]), the desired compound **109** (*m/z* = 446 [$M(^{35}Cl)H^+$], 448 [$M(^{37}Cl)H^+$]) and unreacted alkene **106** (*m/z* = 232 [MH^+]) were observed. This poor selectivity is a general feature in the metathesis reactions of terminal olefin species.[252]



Scheme 3–36: Application of alkene metathesis conditions to alkenes 106 and 107

Unfortunately, this mixture proved to be impossible to separate by silica column chromatography and consequently was taken forward and subjected to reduction using 10% Pd on carbon under H_2 atmosphere, in an attempt to deconvolute the combination of products (Scheme 3–37). This produced an intractable mixture, confirming the unsuitability of this synthetic route for the synthesis of clemastine analogues.



Scheme 3–37: Attempted reduction of metathesis products

Overall, a study into a number of different linking strategies revealed 1,2,3-triazole synthesis and amide bond formation as the most promising candidates for the synthesis of a library of potential *Lmj*IPCS inhibitors based on clemastine **41**. Ester formation was ruled out due to the sensitivity of analogue **96** to degradation in acidic conditions (Scheme 3–32). Additionally, olefin metathesis did not prove to be a viable route, due to poor product selectivity (Scheme 3–36). The analogues that were successfully prepared, namely triazoles **83A** and **83B**, and amides **98** and **99**, were then screened against the *L. major* IPC synthase enzyme and promastigote parasites.

3.3.4 *In vitro* assessment of different linker groups

A primary screen of the analogues synthesised was performed at 20 µM against the *L. major* IPC synthase (Figure 3–6). From the results, it was evident that all *N*-Boc protected analogues

exhibited poor activity. Furthermore, ester **100** was found to be inactive at 20 µM, ruling out esterification as a viable connection strategy.

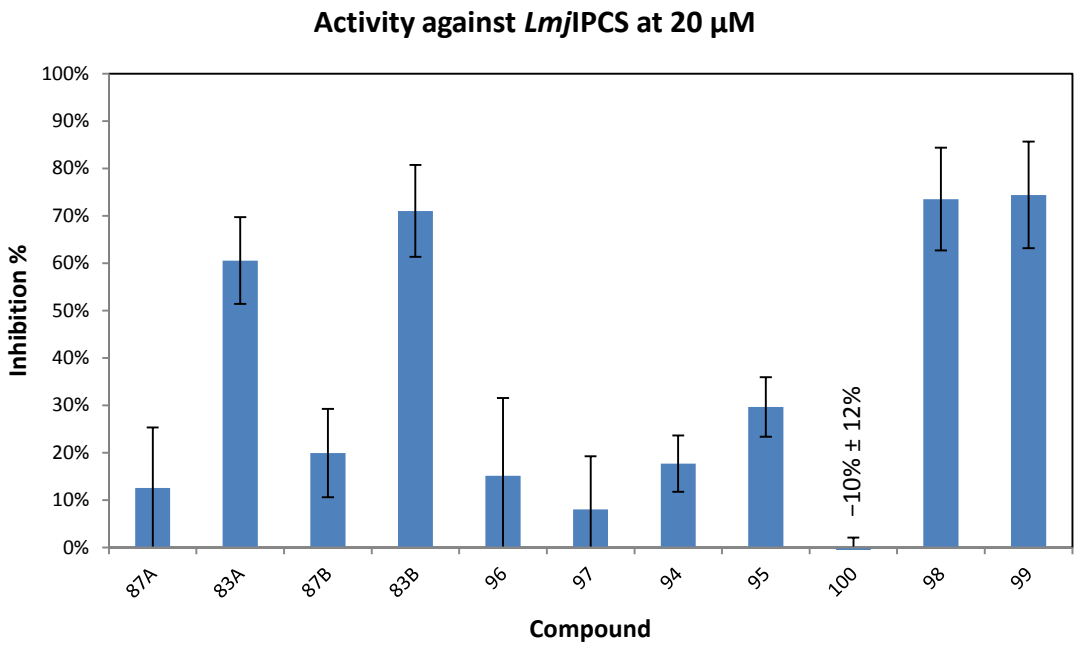


Figure 3–6: Inhibition of *Lmj*IPCS at 20 µM by triazole, amide and ester analogues. Error bars portray standard deviation.

The free amine triazole and amide analogues **83A**, **83B**, **98** and **99** all exhibited reasonable inhibition, and their dose-response relationships were investigated (Figure 3–7 and Figure 3–8). Interestingly, the introduction of rigidity revealed the (*R*,*S*) and (*R*,*R*) diastereomers of triazole mixture **83B** (IC_{50} = 8.49 µM, 95% CI: 7.67 – 9.39 µM) to be 2-fold more potent against *Lmj*IPCS than the (*S*,*R*) and (*S*,*S*) diastereomers of mixture **83A** (IC_{50} = 14.52 µM, 95% CI: 12.39 – 17.02 µM). This observation mirrors the trend of antileishmanial activity observed with nor-clemastine analogues **78A** and **78B** (*c.f.* Figure 3–3). However, in both cases it is unclear whether the observed difference in activity is a general feature of the (2*R*)-pyrrolidine unit, or is the caused by one particular diastereomer. Therefore, it is not possible to state whether there exists a correlation between activity against *Lmj*IPCS and *L. major* promastigotes, particularly, as the nor-clemastine diastereomeric mixtures **78A** and **78B** demonstrated equivalent potency against *Lmj*IPCS (*c.f.* Figure 3–2). Further investigation is required, through the isolation and testing of all diastereomers, before any definitive links may be drawn.

Next, screening against *L. major* promastigotes revealed diastereomeric triazole mixtures **83A** and **83B** to have low leishmanicidal activity, with parasite growth inhibition only

being observed at 100 µM (Figure 3–7). This poor translation of biochemical to antiparasitic activity may be due to decreased cell penetration or solubility imparted by the rigid triazole group.[253]

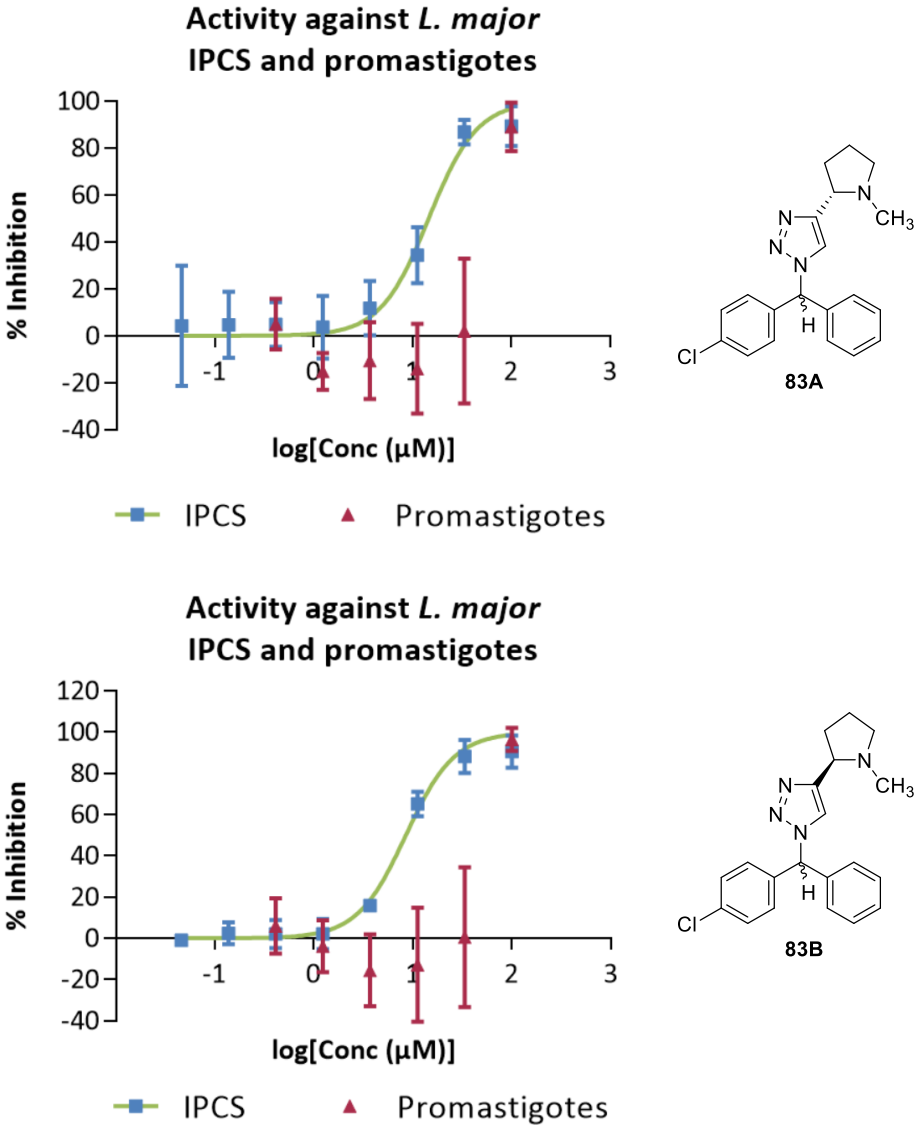


Figure 3–7: Inhibition of *Lmj*IPCS and *L. major* promastigote growth by triazoles 83A and 83B. Error bars portray 95% confidence interval.

Amides **98** and **99** were then tested against the *L. major* IPCS enzyme, revealing respective IC_{50} values of 6.95 µM (95% CI: 6.15 – 7.85 µM) and 6.90 µM (95% CI: 6.10 – 7.80 µM) (Figure 3–8). The reduction in rigidity, compared to triazoles **83A** and **83B**, may be the cause of the observed increase in relative activity. Furthermore, the equivalence of dose-response curves shows that the position of the amide bond is unimportant, indicating that it may not contribute towards any hydrogen bonding interactions with the enzyme. A subsequent enquiry into the activity of amides **98** and **99** against *L. major* promastigotes revealed a lack of

cytotoxic effect until 100 µM (Figure 3–8). As with the triazole analogues **83A** and **83B**, the low observed leishmanicidal activity may be caused by poor cell penetration. In addition, the amide bonds of analogues **98** and **99** may be susceptible to metabolism *via* cleavage by parasite peptidase enzymes.

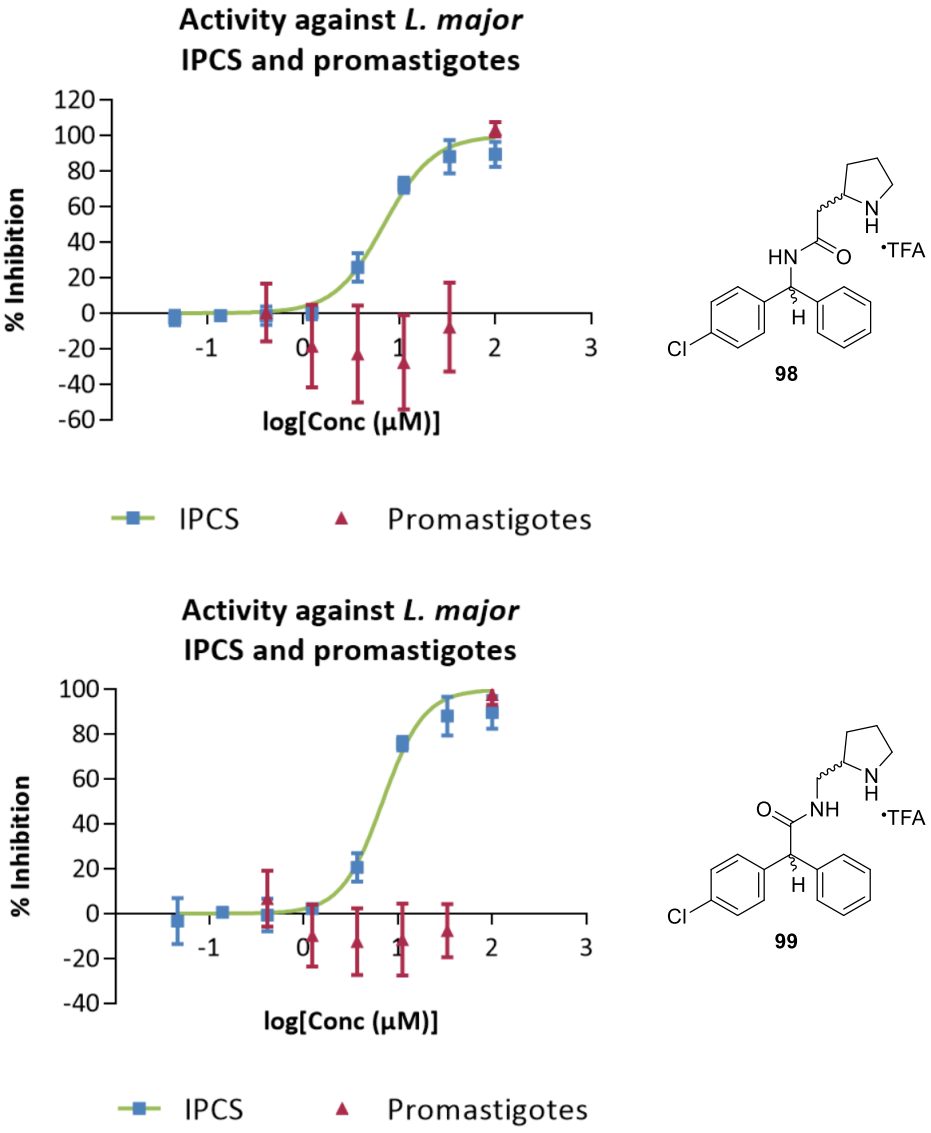


Figure 3–8: Inhibition of *Lmj*IPCS and *L. major* promastigote growth by amides 98 and 99. Error bars portray 95% confidence interval.

3.3.5 Conclusions

A number of desmethyl clemastine compounds, **77**, **78A** and **78B**, were synthesised in order to assess the synthetic tractability of clemastine **41** analogues. Biochemical and cellular screening highlighted that *N*-methylation of the pyrrolidine group improves activity against *L. major* IPC synthase and promastigote parasites. However, as analogues **78A** and **78B** are diastereomeric

mixtures, it is not possible to say whether pyrrolidine stereochemistry or the lack of benzhydryl *C*-methylation, or both, are responsible for the observed decrease in activity of these compounds, relative to clemastine **41**. Interestingly, the higher potency of mixture **78B** over **78A** against *L. major* promastigotes hints at the possible impact of stereochemistry of the pyrrolidine group on leishmanicidal activity. Further testing of the individual diastereomers would be necessary to validate this hypothesis, however. Nevertheless, it was confirmed that a variation in methylation did not result in the complete loss of inhibitory activity.

A following investigation into viable routes for the synthesis of clemastine analogues revealed triazole and amide formation as suitable for the development of IPCS inhibitors. The synthesised analogues were found to be effective against *Lmj*IPCS, but displayed unsuitable activity against *L. major* promastigotes. Of particular interest was the observed higher biochemical activity of triazole analogue mixture **83B**, compared to the complementary (*S*,*S*) and (*S*,*R*) diastereomers in mixture **83A**. The implications of this for antileishmanial activity remain uncertain, however.

These findings come with the caveat that the enantiomeric excesses of the chemical precursors for mixtures **78A**, **78B**, **83A** and **83B** were not measured. As such, the possibility that undesired diastereomers are present in an unknown, albeit likely small, quantity cannot be ruled out. Therefore, the above observations can only be corroborated through isolation and subsequent screening of all possible diastereomers. This is beyond the initial scope of the project, which was to identify suitable connection strategies for the synthesis of clemastine **41** analogues. Because of the observed higher affinity of amide analogues **98** and **99** (compared to triazole analogues **83A** and **83B**) for *Lmj*IPCS and the breadth of potential substrates, peptide bond formation was chosen for the synthesis of a library of small molecule inhibitors based on clemastine **41**.

4. Synthesis of a Small Library of Clemastine Analogues

As discussed in Section 3.1, there was a paucity of SAR data from the primary screen of the NINDS compound set. In addition, inhibitors exhibited narrow chemical diversity. Therefore, a study into the synthesis of a small library of clemastine analogues had two aims:

- Probe the fundamental requirements for activity of clemastine **41** against *Lmj*IPCS
- Explore a more varied chemical landscape for potential inhibitors

Amide bond formation was discovered to be the most promising connection strategy for analogue synthesis (see Section 3.3.4). In addition, IPCS inhibition was not affected by the position of the peptide bond. In collaboration with MRCT Ltd., the project sponsor, an investigation was launched into the synthesis and testing of two series of amide-linked analogues (Figure 4–1). Series 1 would focus on variation of the pyrrolidine moiety of clemastine, whilst the second series would probe the structure-activity relationships of the benzhydryl unit.

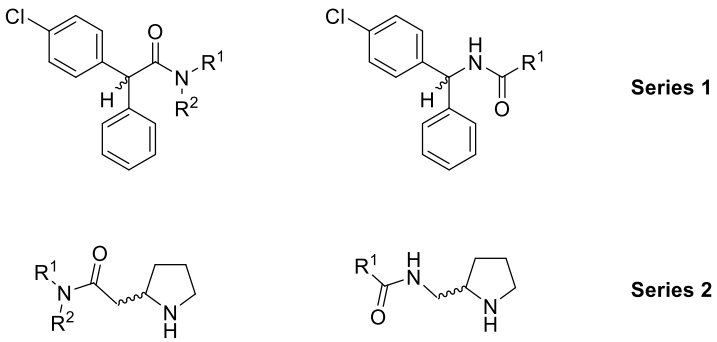


Figure 4–1: Proposed clemastine amide analogue series

4.1 Testing tractability of synthesis

Before embarking on the synthesis of a library of clemastine analogues, preliminary work was required to develop a protocol that would tolerate a wide range of different substrates. First, the procedure employed in the synthesis of amide analogues **94** and **95** was applied to a hindered primary amine **112** and piperazine **113** (Table 4–1, entries 1 & 3).[246]

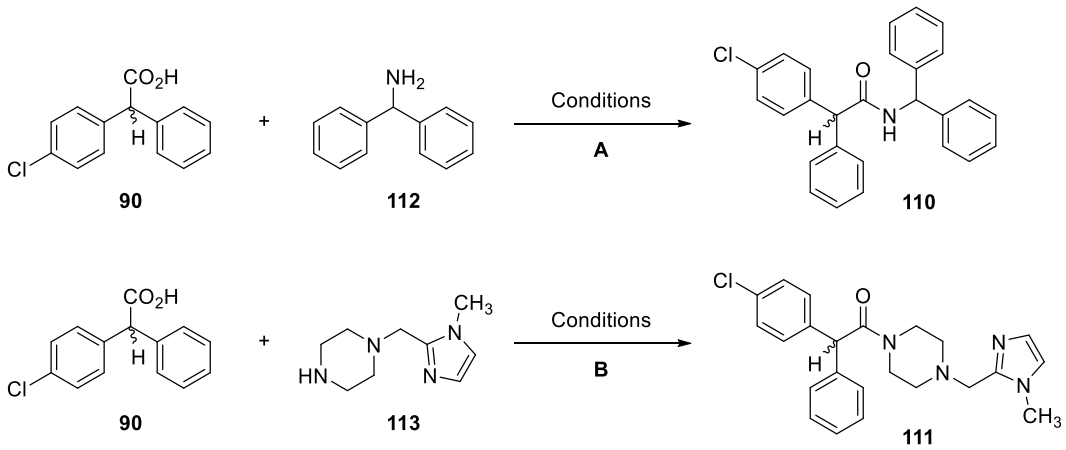
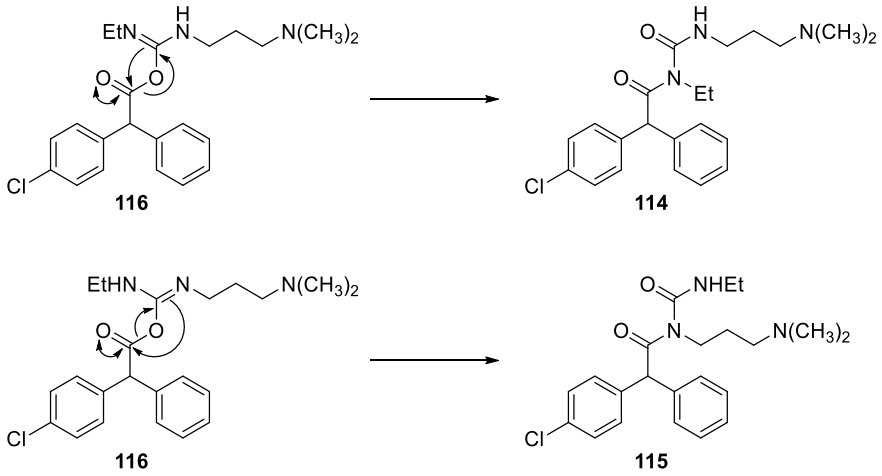


Table 4–1: Synthesis of amides 110 and 111

Entry	Reaction	Conditions	Yield
1	A	EDCI•HCl (1.1 eq.), NMM (1.1 eq.), DCM	0%
2	A	EDCI•HCl (1.1 eq.), HOBt (1.1 eq.), NMM (1.1 eq.), DCM	**110**, 67%
3	B	EDCI•HCl (1.1 eq.), NMM (1.1 eq.), DCM	0%
4	B	EDCI•HCl (1.1 eq.), HOBt (1.1 eq.), NMM (1.1 eq.), DCM	**111**, 37%

Both reactions were found to be unsuccessful, with LCMS analysis of the crude mixtures revealing the presence of a species with *m/z* = 402, consistent with the formation of urea compounds **114** and **115** (Scheme 4–1). The prevalence of this species highlighted that rearrangement of the activated ester **116** to the undesired ureas **114** and **115** was competing with amide formation. To circumvent this issue, a modified procedure was carried out using stoichiometric hydroxybenzotriazole (Table 4–1, entries 2 & 4). LCMS analysis of the reaction mixtures showed disappearance of the postulated urea (*m/z* = 402), allowing the desired amides **110** and **111** to be isolated. Successful coupling reactions were confirmed by the presence of the amide carbonyl stretches at around 1640 cm^{-1} in the IR spectra. This protocol was taken forward and applied to the synthesis of a small library of amide analogues.



Scheme 4–1: Formation of putative urea by-products 114 and 115

4.2 Synthesis of amide library

Peptide coupling reactions were conducted in parallel, in groups of 10 – 20, according to the EDCI and HOBt mediated conditions described above. Briefly, the reaction mixtures were stirred at RT in DCM, until HPLC monitoring of two separate reactions indicated either reagent consumption or lack of progression. The reactions were then quenched with water and passed through hydrophobic phase separators, prior to concentration under a flow of air with heating at 40 °C. If a Boc-deprotection step was required, then the concentrated residues were dissolved in DCM and reacted with TFA at room temperature for 1 hour. After a second concentration step, purification of the crude mixtures was then attempted *via* either normal or reversed-phase flash chromatography, or preparative HPLC.

From a total of 82 reactions, 61 compounds were isolated, highlighting an overall success rate of 74% with an average yield of 37% (see Chapter 8). The majority of failed reactions involved arylamine species which were not commercially sourced and as such may have contained impurities and degradation products. Other compounds proved difficult to purify by both normal and reversed phase flash chromatography and were therefore deemed impractical to pursue.

4.3 *In vitro* testing of compounds

Primarily, the synthesised analogues were screened, in triplicate, against the *L. major* IPC synthase enzyme at a concentration of 20 µM (Figure 4–2) (see Appendix A). The majority of compounds tested were found to have low activity (<20% inhibition).

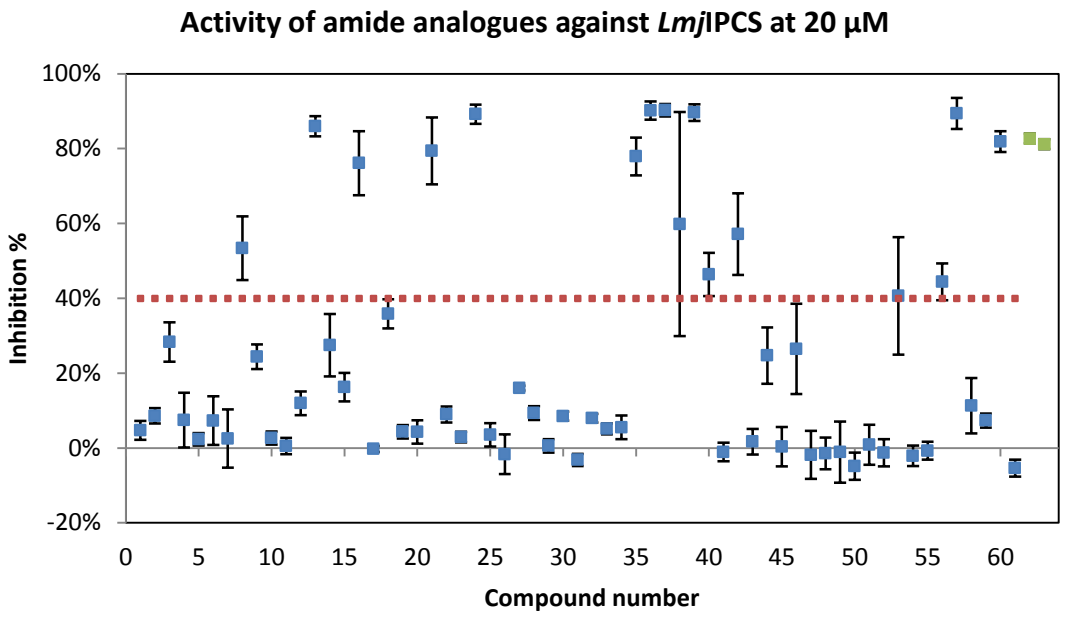


Figure 4–2: Assay of amide library against *Lmj*IPCS at 20 µM. Compound numbers are arbitrarily assigned, relative to their order of testing. 63 Compounds were screened overall, with numbers 62 and 63 (green) representing the parent amides 98 and 99, included as controls. The red dotted line represents the inhibition % value over which compounds were selected for further investigation. Error bars portray standard deviation.

Fifteen compounds that exhibited moderate to high activity (>40% inhibition) against the protozoan enzyme were investigated further through the measurement of their dose-response relationships (Figure 4–3). Compared to clemastine **41** (green bar, IC_{50} = 2.87 µM, 95% CI: 2.25 – 3.66 µM), the majority of compounds displayed low efficacy (IC_{50} >10 µM).

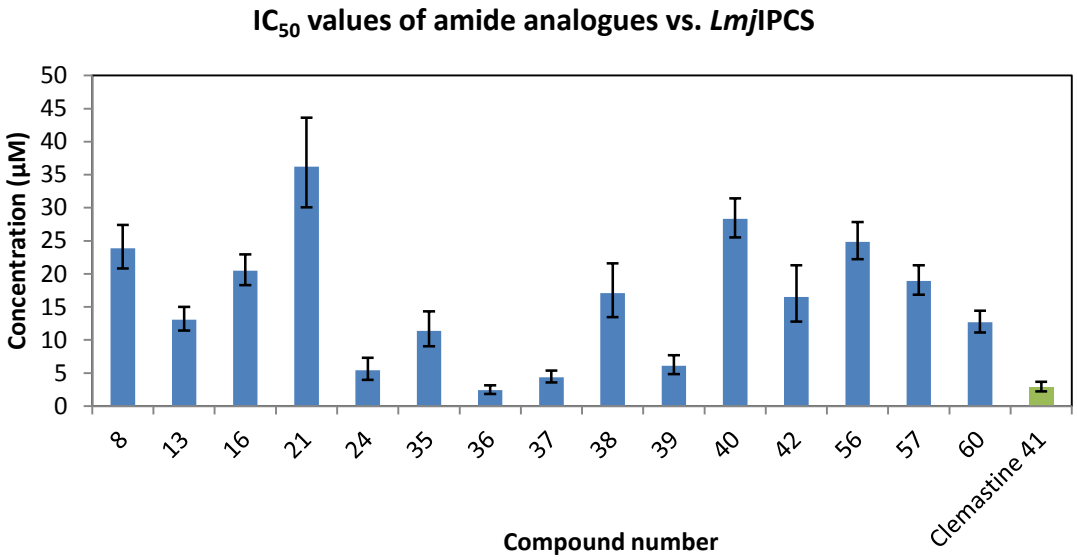
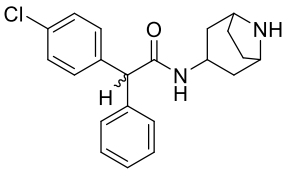
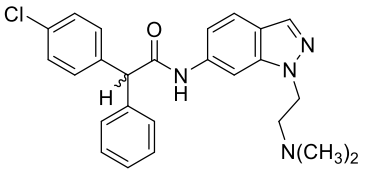


Figure 4–3: IC_{50} measurement of most active amide analogues. Compound numbers correspond to those in Figure 4–2. The final lane (green) represents clemastine 41, included as a control. Error bars portray 95% confidence interval.

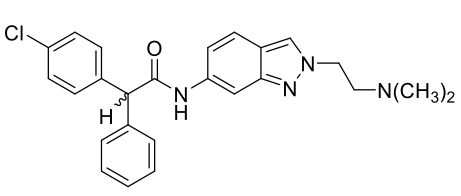
However, amine **117** (Figure 4–3, compound 24), indazoles **118** and **119** (Figure 4–3, compounds 36 and 37), and pyrimidine **120** (Figure 4–3, compound 39) exhibited comparable activity to clemastine **41** (Figure 4–4). Notably, indazole **118** proved to be as active as clemastine **41** against *Lmj*IPCS.



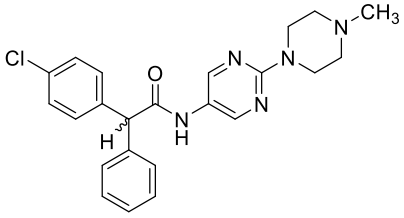
117
IC_{50} = 5.40 μM
95% CI: 3.99 – 7.30 μM



118
IC_{50} = 2.41 μM
95% CI: 1.84 – 3.16 μM



119
IC_{50} = 4.37 μM
95% CI: 3.56 – 5.36 μM



120
IC_{50} = 6.11 μM
95% CI: 4.83 – 7.72 μM

Figure 4–4: The most active IPCS inhibitors from the amide library

Indazole **118** was then tested for parasite toxicity against *L. major* promastigotes, but was only effective above 33 µM (Figure 4–5). As with amide clemastine analogues **98** and **99**, it was postulated that presence of the amide functionality was responsible for the poor translation to antileishmanial activity. In order to test this hypothesis, an analogue of indazole **118** bearing a flexible linker, similar to that of clemastine **41**, would need to be synthesised and screened against both the *L. major* IPCS and promastigote parasites (see Chapter 5).

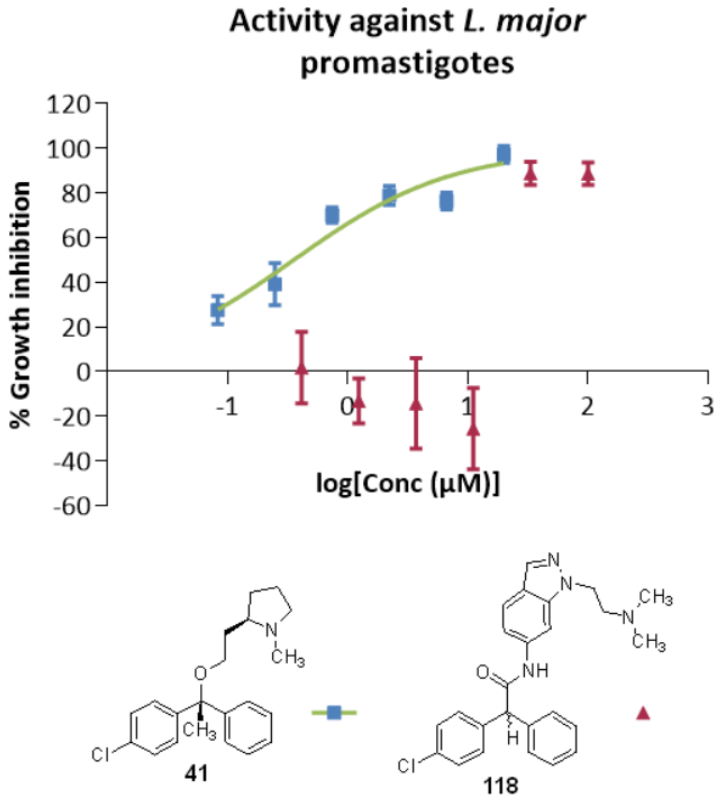


Figure 4–5: Comparison of antileishmanial activity of clemastine 41 and indazole 118. Error bars portray 95% confidence interval.

To conclude, the synthesised library of 61 amide-linked clemastine analogues were assayed against *Lmj*IPCS at 20 µM, revealing the majority (39 compounds, 66%) as poor inhibitors (<20% activity). Fifteen compounds, which inhibited enzyme activity by >40%, were taken forward for dose-response studies against the enzyme, revealing amine **117**, indazoles **118** and **119**, and pyrimidine **120** as the only compounds exhibiting IC_{50} values below 10 µM. Specifically, indazole **118** was found to be as potent as the parent compound, clemastine **41**, although this analogue proved to have poor cytotoxic effect on *L. major* promastigotes.

4.4 Structure-Activity Relationship analysis

A number of structure-activity relationships were drawn from the data introduced above, shedding some light on the activity profile of clemastine **41**.

4.4.1 Amine substituted series

A series of aromatic *N*-heterocycles were screened, including imidazoles, oxazoles, pyrazoles, pyridines, pyrimidines and benzo-fused moieties (Figure 4–6). Low activity was observed at 20 μM with the majority of compounds tested, with the exception of the indazoles **118** and **119**, pyrimidine **120**, imidazole **121** and benzothiazole **122**. Whilst indazoles **118** and **119**, and pyrimidine **120** proved to be effective inhibitors of *Lmj*IPCS (see Figure 4–4), imidazole **121** and benzothiazole **122** displayed significantly higher IC_{50} values (23.89 μM and 36.21 μM respectively).

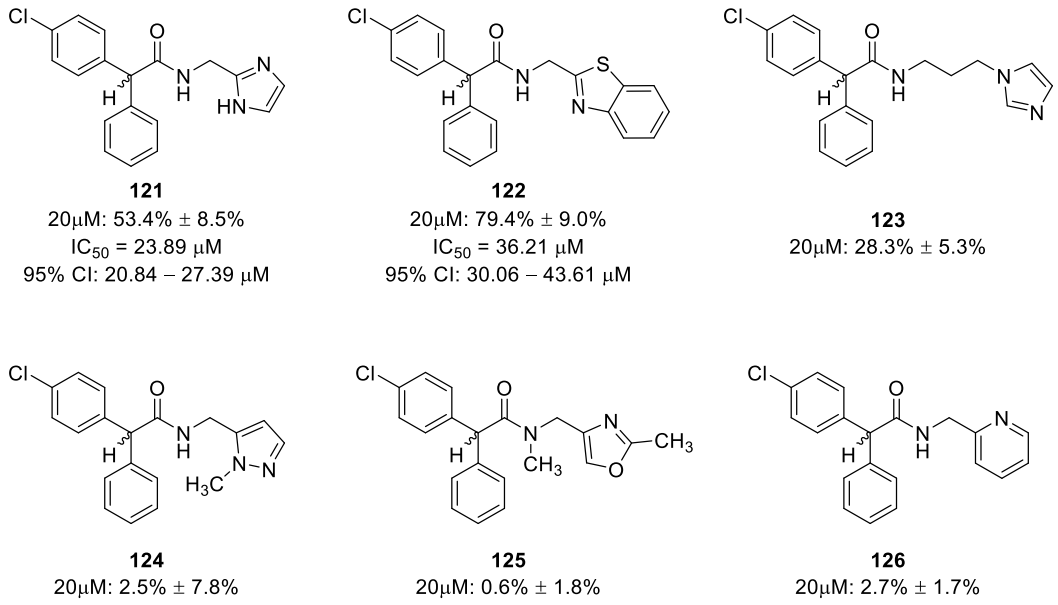
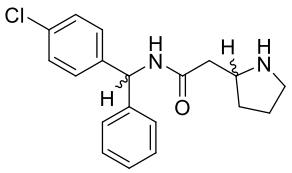


Figure 4–6: Example aromatic *N*-heterocyclic analogues tested

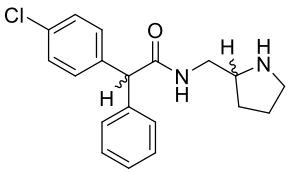
The indazoles **118** and **119**, and pyrimidine **120** all feature a terminal aliphatic amine moiety, whereas imidazole **121** and benzothiazole **122** do not. This may be important for activity, although further experiments would be required to make a direct comparison. Indeed, the higher activity of 1-substituted indazole **118** over 2-substituted indazole **119** indicates that the placement of the dimethylamino group has an effect on potency (Figure 4–4).

A study into aliphatic substituted analogues provided further insight (Figure 4–7, Figure 4–8 and Figure 4–9). Azetidine analogues **127** and **128** proved effective in the primary

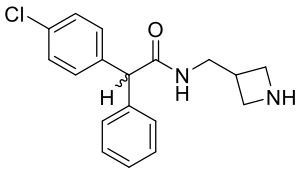
20 μM screen, but displayed lower potency compared to the parent amides **98** and **99**. Similarly, pyrrolidine **129** exhibited a relatively high IC_{50} value (20.49 μM). This observation may be explained by the position of the nitrogen atom. For example, the amine moiety of analogues **127**, **128** and **129** is located 4 bonds away from the amide bond, whilst 3 bonds separate the pyrrolidine nitrogen of parent analogues **98** and **99**. A more detailed study into pyrrolidine containing analogues of varying chain length is required to verify this hypothesis.



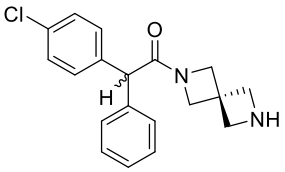
98
20μM: 82.7% ± 1.3%
IC_{50} = 6.95 μM
95% CI: 6.15 – 7.85 μM



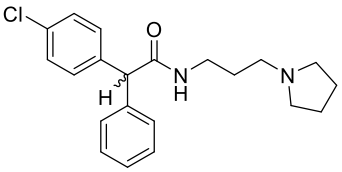
99
20μM: 81.2% ± 1.3%
IC_{50} = 6.90 μM
95% CI: 6.10 – 7.80 μM



127
20μM: 86.0% ± 2.7%
IC_{50} = 13.09 μM
95% CI: 11.43 – 15.00 μM



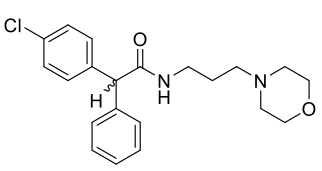
128
20μM: 77.9% ± 5.1%
IC_{50} = 11.38 μM
95% CI: 9.03 – 14.34 μM



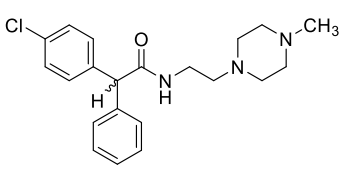
129
20μM: 76.1% ± 8.5%
IC_{50} = 20.49 μM
95% CI: 18.30 – 22.94 μM

Figure 4–7: Pyrrolidine and azetidine analogues

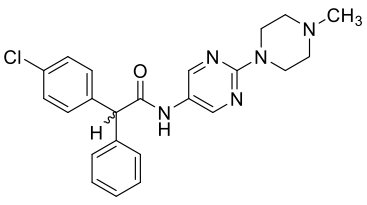
Morpholine and piperazine analogues, such as compounds **130** and **131**, fared poorly against the IPCS enzyme (Figure 4–8). It is worth noting, however, that the pyrimidine analogue **120** (89.6% inhibition at 20 μM), which contains an *N*-methylpiperazine unit, exhibits higher activity than the aliphatic analogue **131** (16.3% inhibition at 20 μM). The higher affinity of analogue **120** may be due to the pyrimidine unit, or the increased distance between the benzhydryl and piperazine moieties.



130
20μM: 35.8% ± 3.9%



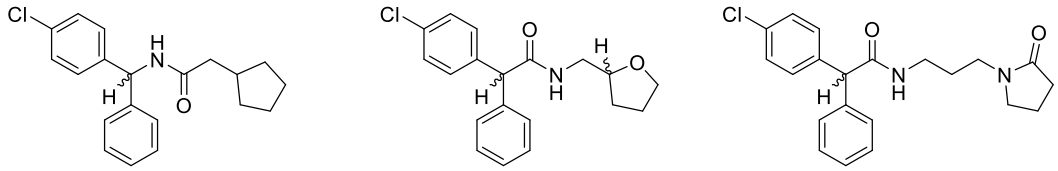
131
20μM: 16.3% ± 3.8%



120
20μM: 89.6% ± 2.2%

Figure 4–8: Morpholine and piperazine analogues

An important conclusion was drawn from cyclopentyl and tetrahydrofuranyl analogues **132** and **133** (Figure 4–9). Their lack of activity at 20 μM, compared to pyrrolidine analogues **98** and **99** (*c.f.* Figure 4–7), highlighted the necessity of nitrogen-containing functionality. The poor activity of tetrahydrofuran **133** also indicated that H-bond acceptor capability may not be important for inhibition. Furthermore, a comparison between pyrrolidine **129** and lactam **134** reveal that IPCS inhibition relies on basic amino moieties. Drawing a parallel to the ceramide analogue work by Mina,[190] the presence of a protonated nitrogen species may interfere with the active site, bringing about inhibition of IPC synthesis (see Section 1.3.4.4).



132
20μM: –5.4% ± 2.3%

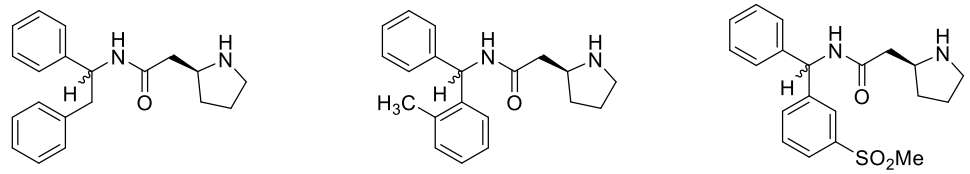
133
20μM: 2.9% ± 1.4%

134
20μM: –0.3% ± 0.8%

Figure 4–9: Example aliphatic analogues tested

4.4.2 Benzhydryl substituted series

A number of analogues with bis-aryl functionality were explored (Figure 4–10). Compared to the parent amide **98** (82.7% inhibition at 20 μM), increasing rotational freedom *via* insertion of a methylene linker resulted in a dramatic drop in IPC synthase inhibition at 20 μM (compound **135**, 44% inhibition), whilst an attempt to restrict rotation, in the case of amine **136**, maintained activity (89% inhibition). However, dose-response measurements revealed both amides **135** and **136** to be comparatively weak inhibitors.



135
20μM: 44.4% ± 4.9%
IC_{50} = 24.86 μM
95% CI: 22.22 – 27.82 μM

136
20μM: 89.4% ± 4.1%
IC_{50} = 18.94 μM
95% CI: 16.83 – 21.32 μM

137
20μM: 11.3% ± 7.4%

Figure 4–10: Example bis-aryl analogues

A brief exploration into aryl substituents saw electron deficient amine **137** exhibit poor inhibition at 20 μM. A complementary analogue bearing a *para*-methoxy substituent could not be purified and hence was not tested. In addition, there remains the caveat to these findings

that the compounds **135**, **136** and **137** were tested as diastereomeric mixtures, which may account for an as yet unknown effect on potency against the *Lmj*IPCS enzyme.

Replacement of one of the aryl groups revealed a preference for long-chain fatty residues (Figure 4–11). Testing of analogues **138** and **139** against *Lmj*IPCS at 20 μM revealed a complete loss in activity upon replacement with a hydrogen or methyl unit. By contrast, amide **140** was found to be very potent at 20 μM, though determination of an IC_{50} value revealed lower efficacy, compared to parent amides **98** and **99** (*c.f.* Figure 4–7). Furthermore, it was found that *n*-pentyl substitution was preferred over cyclopentyl (analogue **141**, IC_{50} = 28.31 μM), with cyclohexyl substituted amide **142** exhibiting even lower activity (24.7% inhibition at 20 μM).

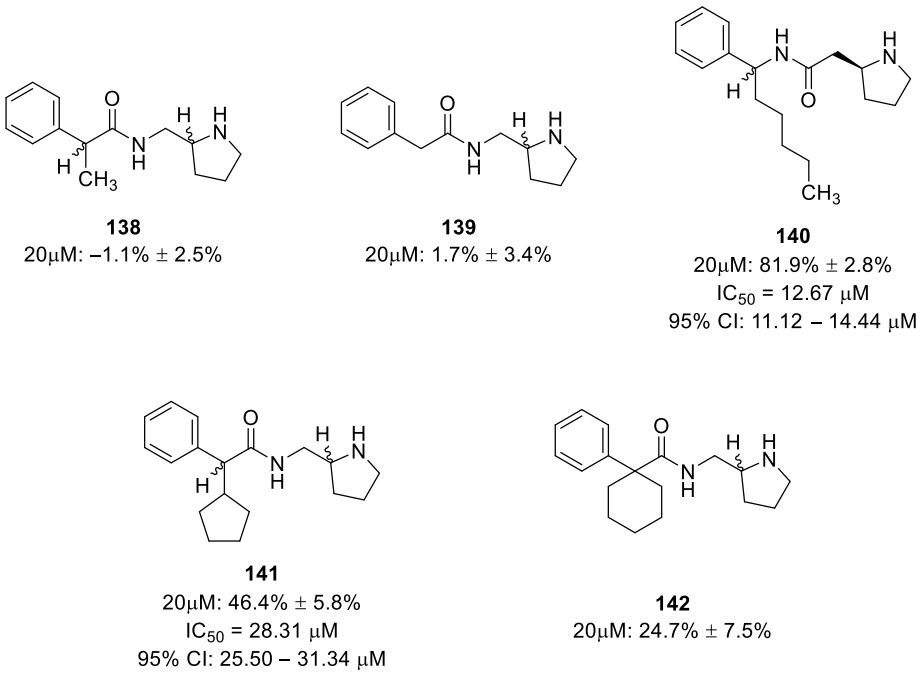
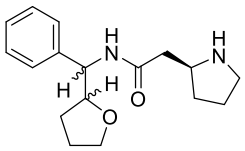


Figure 4–11: Mono-aryl amide analogues

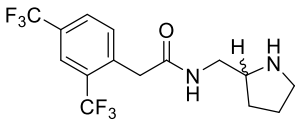
Of the other analogues tested, a notable comparison of cyclopentyl and tetrahydrofuranyl analogues **141** and **143** revealed a potential low tolerance to the presence of heteroatoms (Figure 4–12). Additionally, bis-trifluromethyl benzyl amide **144** displayed a significant increase in potency over the related benzyl compound **139**. Incorporation of the trifluoromethyl group into other analogues of clemastine **41** may furnish an increase in activity against *Lmj*IPCS.

The discovery of higher activity with analogues bearing both aliphatic and aryl substituents points towards potential hydrophobic and π-stacking interactions. Alternatively,

the observed potency may reflect a higher concentration of these compounds near the lipid-bound *Lmj*IPCS.



143
20μM: 7.3% ± 1.9%



144
20μM: 57.2% ± 10.9%
IC_{50} = 16.51 μM
95% CI: 12.80 – 21.29 μM

Figure 4–12: Other substituted benzyl amides

4.5 Conclusions

A library of 61 clemastine analogues was synthesised utilising a straightforward peptide coupling strategy. Subsequent screening of these analogues revealed indazole **118** to be as effective as clemastine **41** against *Lmj*IPCS. However, this analogue exhibited low activity against *Leishmania major* promastigotes, possibly due to the presence of amide bond functionality.

A number of important SAR discoveries were made from the biochemical screening data gathered. These findings are summarised below, alongside the features learned from Chapter 3 (Figure 4–13).

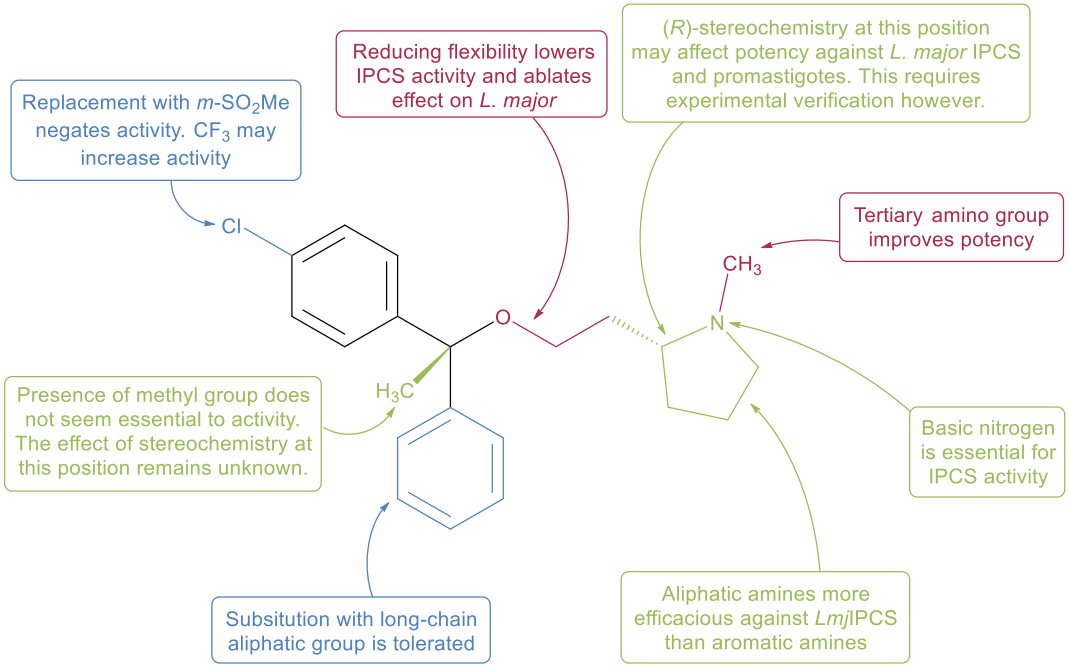


Figure 4–13: Key SAR observations

One of the more notable discoveries in this investigation was the requirement of basic amino functionality for IPCS inhibition. Additionally, studies on the benzhydryl portion of the molecule revealed a preference for large hydrophobic substituents.

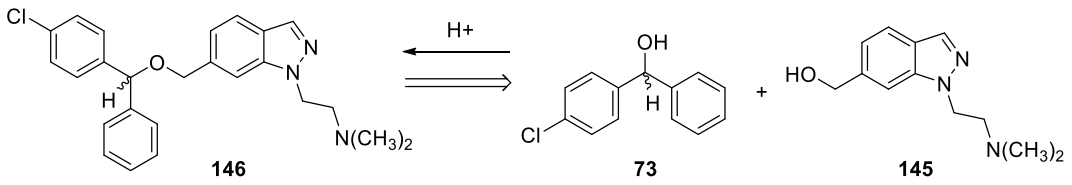
The SAR data gained have highlighted some key areas for further investigation. A study of analogues bearing a range of aryl-substituents will provide in-depth information on the interactions of the benzhydryl unit with the IPC synthase enzyme, shedding light on potential π-stacking and hydrophobic interactions. In addition, altering the linker chain length, as well as the position of the nitrogen on the pyrrolidine ring, may reveal different activity profiles. This in turn could point towards specific interactions with the *Lmj*IPCS enzyme. More fundamentally, as reflected in Chapter 3, an in-depth investigation into the effects of pyrrolidinyl and benzhydryl stereochemistry on potency against both *L. major* IPCS and promastigote parasites may consolidate some of the patterns discovered in this work.

5. Efforts Towards the Development of a Second Generation Inhibitor

As described in Section 4.3, indazolyl amide **118** was discovered to have activity against *Lmj*IPCS at concentrations comparable to clemastine **41**, but exhibited poor antileishmanial action. This observation mirrored the low cytotoxic activity displayed by clemastine amide analogues **98** and **99** (see Section 3.3.4). Therefore, it was decided to synthesise an ether-linked analogue of indazole **118**, to test whether presence of the amide bond was responsible for this differential action.

5.1 Synthetic strategy

As with the synthesis of nor-clemastine analogues **77**, **78A** and **78B** (see Section 3.2.2), it was postulated that alcohols **73** and **145** could be connected *via* acid catalysed ether formation to access indazole analogue **146** (Scheme 5–1). With benzhydrol **73** readily available, attention turned towards the synthesis of fragment **145**.

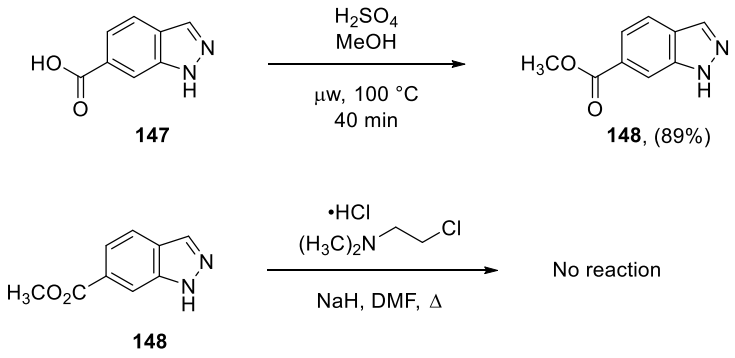


146 **73** **145**

Scheme 5–1: Retrosynthetic analysis of indazole 145

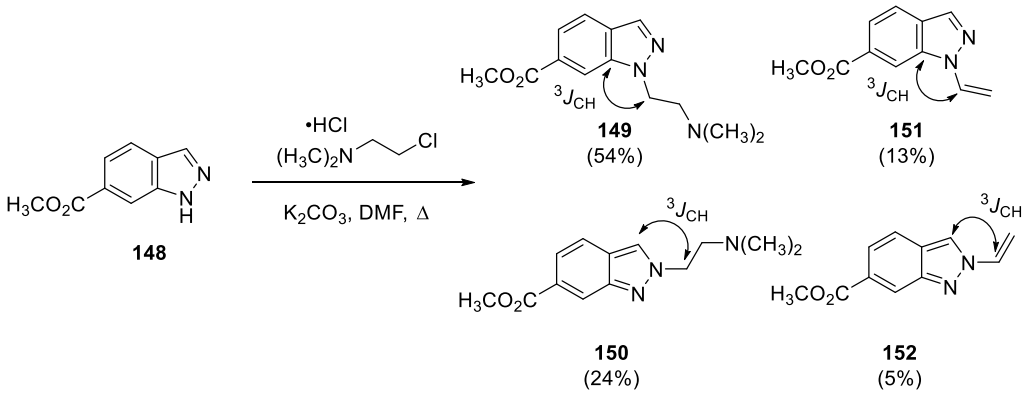
5.2 Synthesis of indazolyl methanol 145

Indazole-6-carboxylic acid **147** was converted to the methyl ester **148** *via* acid catalysed esterification, as confirmed by the appearance of the molecular ion in the LCMS spectrum (*m/z* = 177 [MH^+]) (Scheme 5–2).[254] Initial attempts to alkylate the indazole utilising NaH and 2-chloro-*N*,*N*-dimethylethylamine (as a free base) resulted in no conversion of ester **148**, as judged by TLC analysis.[255]



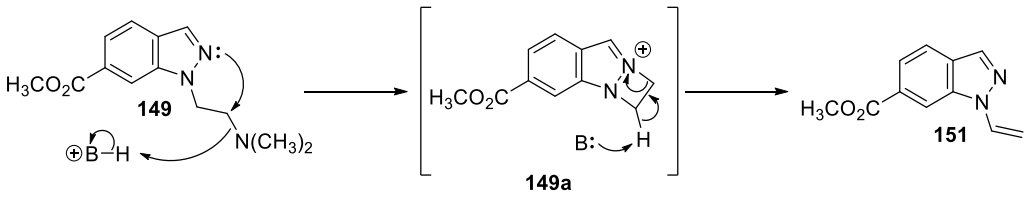
Scheme 5–2: Esterification and attempted *N*-alkylation of indazole-6-carboxylic acid 147

Alternative conditions, using potassium carbonate as base, resulted in the full consumption of ester **148**, but provided 4 compounds as observed by TLC and LCMS analysis of the crude reaction mixture (*m/z* = 203 and 248) (Scheme 5–3). Straightforward separation on SiO_2 column afforded *N*-alkylation products **149** and **150**, as well as *N*-vinyl indazoles **151** and **152**. Characterisation of the separate isomers was confirmed through analysis of 2D NMR HMBC correlations (shown below). Specifically, the desired product **149** revealed mutual coupling between the quaternary carbon at δ_C = 139.2 ppm and the methylene unit at δ_H = 4.52 ppm.



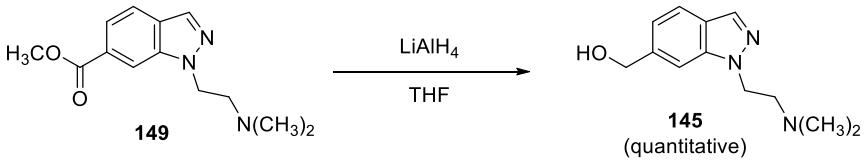
Scheme 5–3: Alkylation of ester 148 under K_2CO_3 conditions

The mechanism by which elimination products **151** and **152** were formed was not investigated, but may have involved anchimeric assistance, as exemplified by the putative intermediate **149a** (Scheme 5–4). An optimisation of this alkylation step was not pursued, as the difficulty of achieving exclusive 1-*N* or 2-*N* alkylation with indazoles is well documented.[255–257]



Scheme 5–4: Possible elimination mechanism for the formation of olefin 151

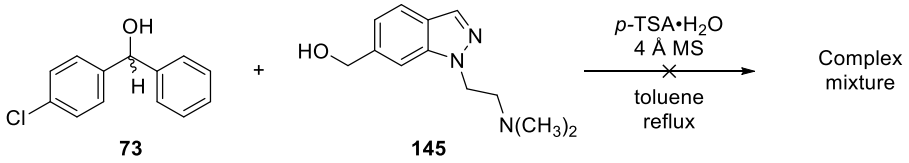
Hydride reduction of the ester moiety of indazole **149** resulted in clean conversion to alcohol **145**, as evidenced by the disappearance of the IR-active carbonyl stretch and appearance of a broad O–H vibration at 3348 cm^{-1}. Indazole **145** proved to be unstable to chromatography on silica and so was used without purification.



Scheme 5–5: Ester reduction of indazole 149

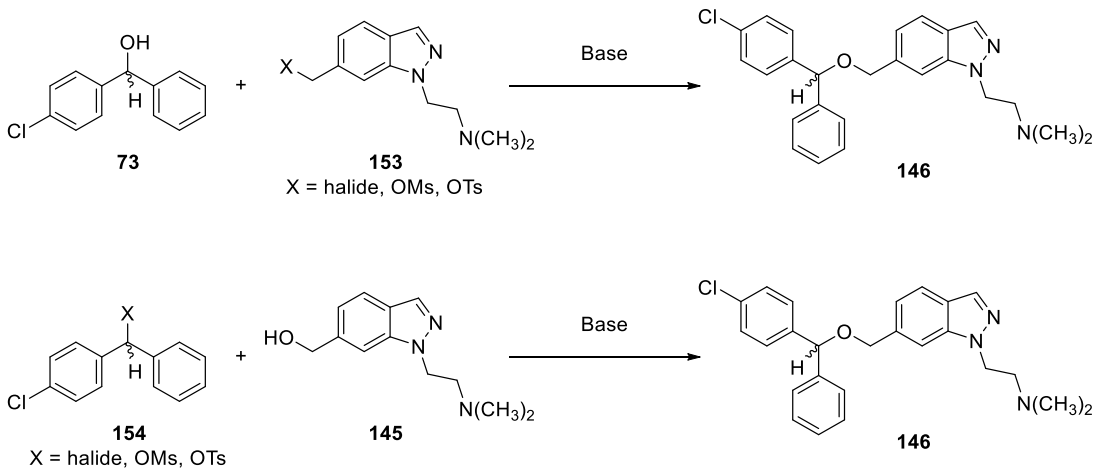
5.3 Ether formation

Initial attempts to couple benzhydrol **73** and indazole **145** followed the acid catalysis protocol employed for the synthesis of nor-clemastine analogues **77**, **78A** and **78B** (see Section 3.2.2) (Scheme 5–6). However, an intractable mixture resulted, probably reflecting the instability of the indazole alcohol **145**.



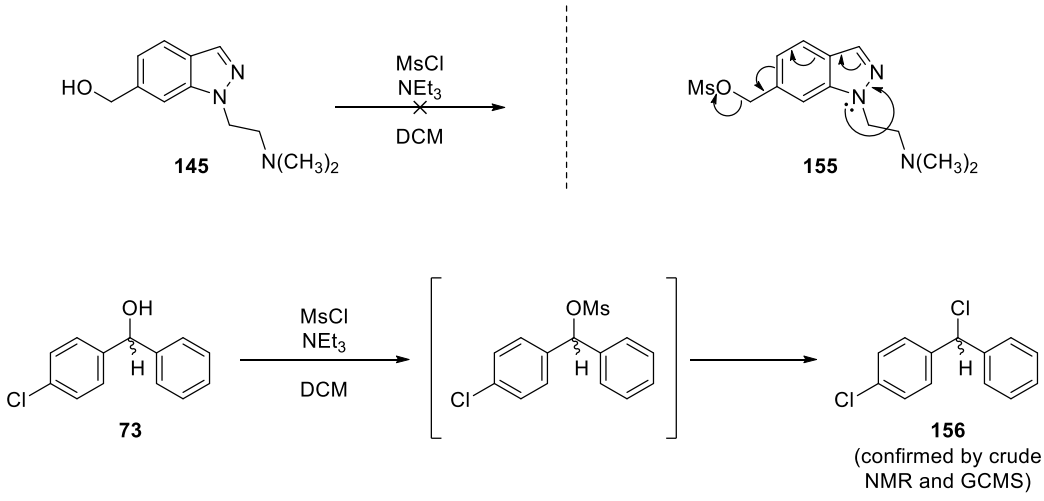
Scheme 5–6: Failed ether formation attempt with alcohols 73 and 145

An alternative strategy for the preparation of the desired analogue **146**, through Williamson-type ether synthesis, was then investigated, beginning with an exploration of the synthesis of alkylating agents **153** and **154** (Scheme 5–7).



Scheme 5–7: Displacement strategy for the synthesis of indazole 146

Initial attempts examined the reaction of indazole **145** with mesyl chloride (Scheme 5–8). Although consumption of the starting material was observed on TLC analysis of the reaction mixture, upon workup of the reaction, a complex mixture resulted from which the desired mesylate **155** could not be obtained. It is speculated that this may be due to participation of the indazole nitrogen, activating the putative mesylate **155** towards further reaction. As it was previously demonstrated that benzhydrol **73** could be activated using mesyl chloride (see Scheme 3–35), the conversion of alcohol **73** to a suitable electrophile was then explored.

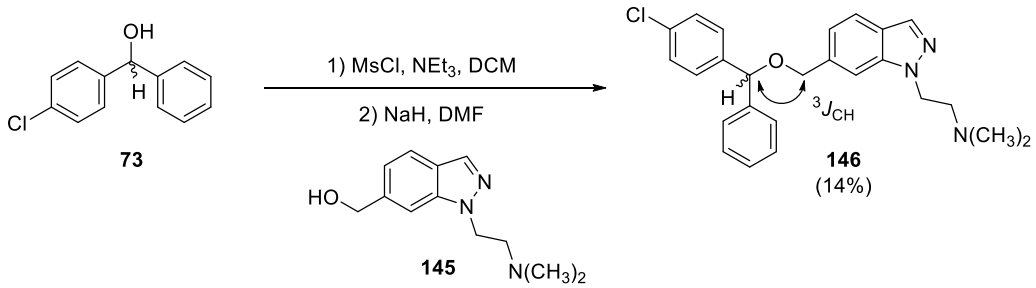


Scheme 5–8: Attempted activation of alcohols 73 and 145

The reaction of benzhydrol **73** with methanesulfonyl chloride proceeded cleanly, as judged by TLC analysis (Scheme 5–8). After washing the resulting crude product with $NaHCO_{3(aq.)}$, GCMS analysis revealed the presence of chloride **156**, displaying a characteristic Cl_2 isotopic

pattern (*m/z* = 236 [100%, M(^{35}Cl)(^{35}Cl)], 238 [83, M(^{35}Cl)(^{37}Cl)], 240 [8, M(^{37}Cl)(^{37}Cl)]). This was in agreement with the crude ^{1}H NMR spectrum, where no methyl group was observed, presumably due to removal of methanesulfonic acid (δ_H ~2.80 ppm). Unfortunately, an attempt to purify chloride **156** *via* SiO_2 column resulted in the complete loss of material.

With the knowledge that aryl chloride **156** could be accessed, an attempted two-step reaction was conducted with activation of benzhydrol **73** prior to displacement by the alkoxide of indazole **145** (Scheme 5–9). The desired ether **146** was isolated in 14% yield after chromatography on silica, confirmed by a 2D NMR HMBC experiment, which revealed coupling between the depicted methine (δ_H = 5.45 ppm, δ_C = 82.1 ppm) and methylene (δ_H = 4.68 ppm, δ_C = 71.0 ppm) units.



Scheme 5–9: Synthesis of indazolyl ether 146

5.4 *In vitro* activity of ether analogue 146

With the desired ether **146** in hand, a dose-response assay against the *L. major* IPC synthase enzyme was conducted (Figure 5–1). Surprisingly, the synthesised analogue **146** was found to have a lower IC_{50} value (4.72 µM, 95% CI: 3.90 – 5.72 µM), compared to the parent indazole amide **118** (*c.f.* Figure 4–4, 2.41 µM, 95% CI: 1.84 – 3.16 µM). The reason for this discrepancy is unclear, but may be due to the loss of H-bond donor/acceptor capability of the amide, or a loss of conformational rigidity.

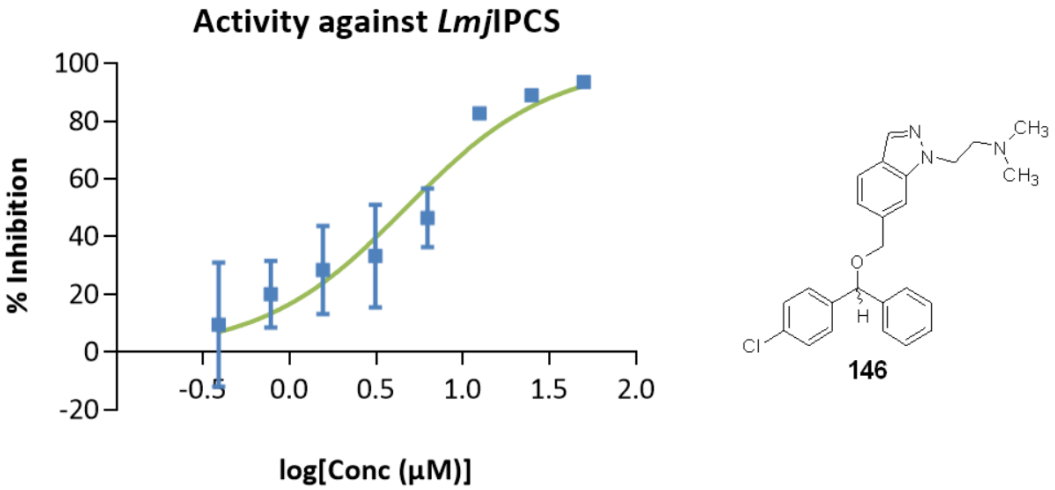


Figure 5–1: Dose-response relationship of indazole 146 against *Lmj*IPCS. Error bars portray 95% confidence interval

Subsequently, cell-viability assays against *L. major* promastigotes were undertaken. Whilst ether **146** saw a modest improvement in activity when compared to the parent amide **118** (Figure 5–2), the activity was considerably lower (ED_{50} >5 µM) than that observed for clemastine **41** (ED_{50} = 0.47 µM).

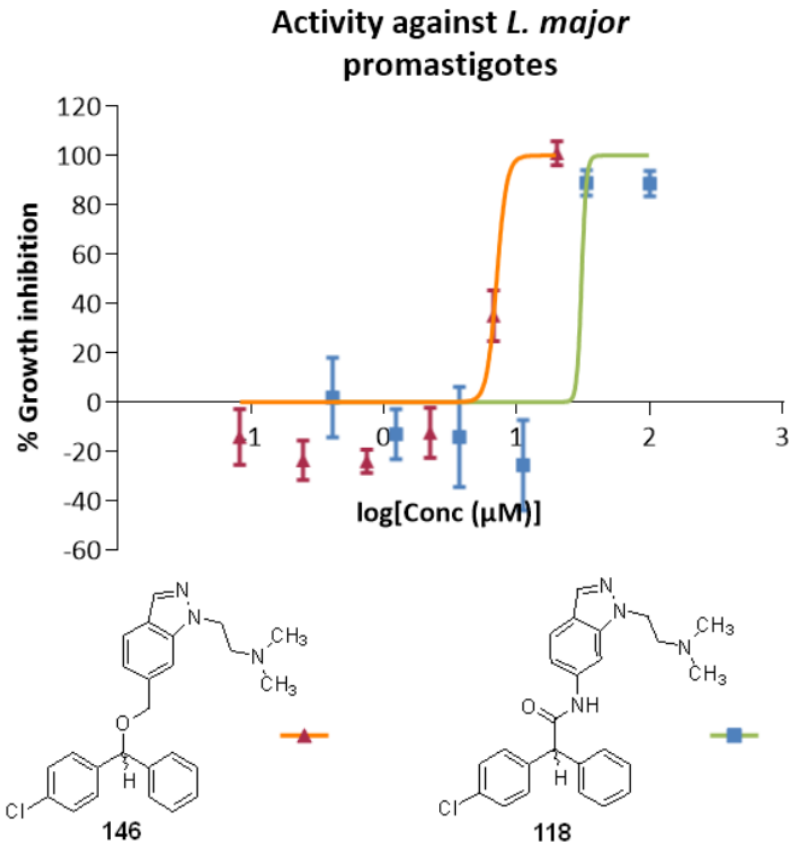


Figure 5–2: Compared activity of indazoles 118 and 146 against *L. major* promastigotes. Error bars portray 95% confidence interval.

The reasons for the poor leishmanicidal activity of indazole **146** remain unknown, but may be due to poor cell permeability, metabolism by the parasite, or an inability to reach the target IPCS. However, the increase in antileishmanial activity upon removal of the amide bond is consistent with the observation that the diastereomeric mixture **77** exhibits micromolar leishmanicidal action, unlike amide analogues **98** and **99**, which also contain a mixture of 4 diastereomers (*c.f.* Figure 3–3 and Figure 3–8).

5.5 Conclusions

Based on the most active *Lmj*IPCS inhibitor from the library of amide-linked compounds, indazole **118** (see Figure 4–4), the ether-linked analogue **146** was prepared, in order to assess the effect of the amide bond on leishmanicidal activity. Although the synthesised ether **146** was found to be a slightly less effective inhibitor of *Lmj*IPCS, it displayed higher potency against *L. major* promastigotes. Nevertheless, ether **146** proved to be much less effective than clemastine **41** against the flagellate parasites. This verifies that removal of the amide functionality allows for an increase in antileishmanial activity, but is not the only factor affecting compound efficacy.

As observed with other target-based screening projects, biochemical activity does not necessarily translate to efficacy *in cellulo* (see Section 1.2.5.2).[115] However, the work presented in this thesis has established an iterative method of analogue synthesis and *in vitro* screening, to allow for the straightforward elucidation of SAR data. This in turn will inform the generation of more active chemical tools to further probe the *Leishmania major* IPC synthase enzyme.

6. Additional Work

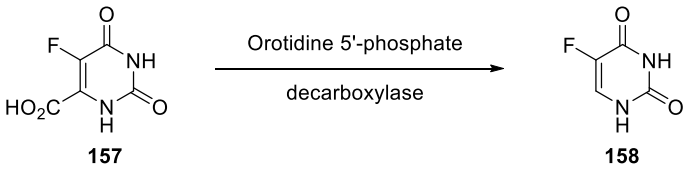
This chapter covers additional work that was conducted to support the main investigation discussed throughout Chapters 2 – 5. Section 6.1 discusses the construction of a mutant yeast strain that was required to effectively produce microsomal material enriched in the *Lmj*IPCS enzyme. This work was conducted collaboratively within the Denny group, and due credit is given where appropriate.

Section 6.2 introduces efforts made towards developing a photoaffinity probe that may potentially shed light on the interactions of clemastine **41** analogues with the *Lmj*IPCS enzyme. All work conducted in this section is the author's own.

6.1 Construction of a *Lmj*IPCS complemented mutant yeast strain

The yeast system initially used to probe the activity of *Lmj*IPCS (YPH499–HIS–GAL–AUR1 pRS246–*Lmj*IPCS, see Section 1.3.4.4) produces *Lmj*IPCS, but not the fungal *S. cerevisiae* IPCS (AUR1), in the presence of glucose.[159,209] However, concerns over the potential for leaky expression of *Sc*AUR1 were raised by Sevova *et al.*[258] To address this concern, work within the Denny group focussed on the generation of a yeast knock-out mutant which lacked the *AUR1* gene. As *S. cerevisiae* requires a functional IPCS to survive,[192] transformation with the exogenous *Lmj*IPCS gene would rescue the mutant yeast.

To this end, an auxotrophic Δ*AUR1 S. cerevisiae* strain was utilised (α *ade*$^-$ *lys*$^-$ *leu*$^-$ *AUR1Δ::TRP1* pRS316–Sc*AUR1*), in which the genomic *AUR1* was replaced by a *TRP1* gene, encoding *de novo* tryptophan biosynthesis.[259] The mutant yeast was rescued by inclusion of a pRS316 plasmid containing the essential *AUR1* enzyme. This plasmid also contains a *URA3* gene, which encodes the orotidine 5'-phosphate decarboxylase enzyme, required for uracil production.[260] Presence of the *URA3* gene confers a sensitivity to 5-fluoroorotic acid **157** (5-FOA), which is converted to toxic 5-fluorouracil **158** by orotidine 5'-phosphate decarboxylase (Scheme 6–1). The use of 5-FOA **157** is a well-established strategy for the counter-selection of genes in yeast.[261]



Scheme 6–1: Biosynthesis of 5-fluorouracil 158, catalysed by orotidine 5'-phosphate decarboxylase

The *Leishmania major* IPCS and *Sc*AUR1 coding sequences were cloned into separate pESC–LEU expression vectors, bringing the expression of these open reading frames under the control of a *GAL1* promoter, meaning the respective IPC synthases are expressed in the presence of galactose but suppressed by glucose (Figure 6–1). The resulting pESC–LEU–*LmjIPCS* and pESC–LEU–*ScAUR1* plasmids were subsequently used to transform the Δ*AUR1* yeast strain, which was then subjected to 5-FOA **157** selection conditions.

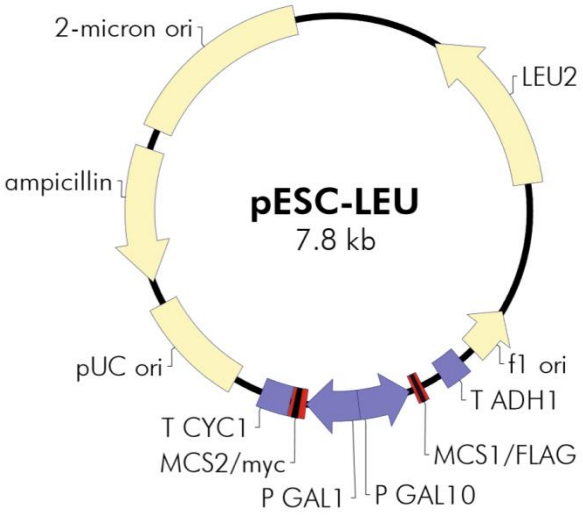


Figure 6–1: The pESC–LEU vector. Multiple cloning sites (MCSs) are under *GAL* promotion, meaning gene expression is activated by galactose and repressed by glucose. The presence of the *LEU2* marker allows for growth of transformed yeast in media lacking leucine.

These selection conditions successfully cured the transgenic yeast of the pRS316–Sc*AUR1* plasmid, which was confirmed in a complementation assay (Figure 6–2). The transformed Δ*AUR1* pESC–LEU–*LmjIPCS* and Δ*AUR1* pESC–LEU–*ScAUR1* yeast strains were found to grow on galactose containing medium (SGR−W−L), under which IPCS expression is activated. Furthermore, the lack of growth on glucose containing medium (SD−W−L), where expression from the pESC–LEU plasmid is suppressed, proves the absence of the pRS316–Sc*AUR1* plasmid.

Lastly, no yeast growth was observed on medium containing galactose but lacking uracil (SGR−W−L−URA), indicating the absence of the *URA3* gene, and hence pRS316–Sc*AUR1*. This experiment confirms the construction of a mutant yeast strain complemented with the *Lmj*IPCS enzyme as its only functional sphingolipid synthase.

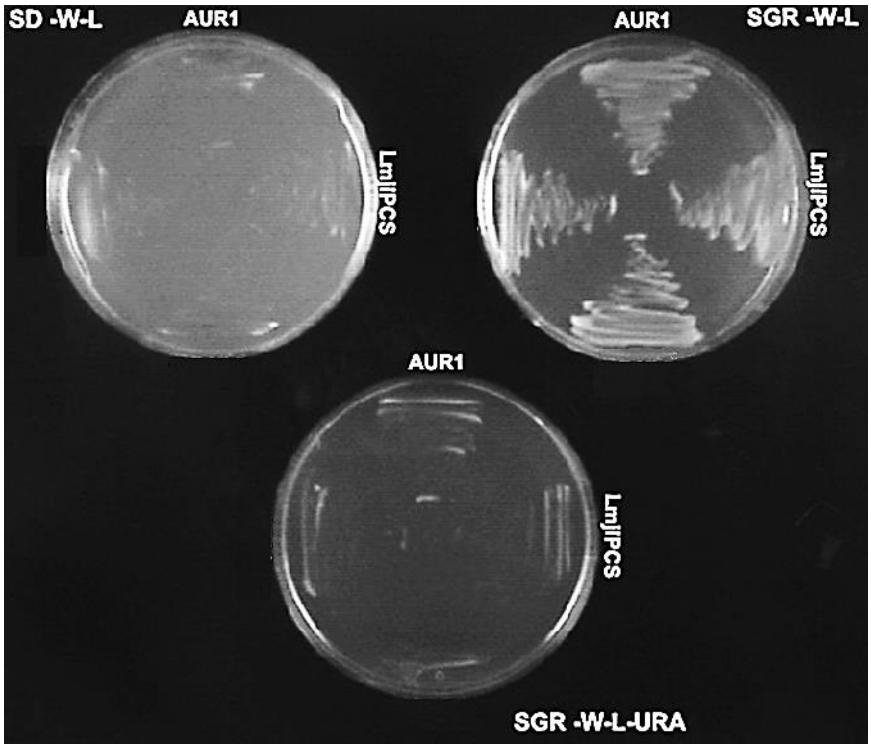


Figure 6–2: Complementation assay of Δ*AUR1* pESC–LEU–*LmjIPCS* and Δ*AUR1* pESC–LEU–*ScAUR1 S. cerevisiae* strains. Assay conducted by J.L. Norcliffe (data unpublished). Minimal media are lacking either tryptophan (−W), leucine (−L) or uracil (−URA), and with either glucose (SD) or galactose/raffinose (SGR) as carbon sources.

Following this, the function of the IPC synthases within these yeast strains was confirmed *via* an *ex cellulo* experiment (Figure 6–3). Microsomal membranes were harvested from the mutant yeast and used to catalyse the reaction of fluorescent NBD-C_6-ceramide and phosphatidylinositol (PI). The resulting mixture of ceramide and NBD-C_6-IPC **35** was separated by HPTLC and visualised through fluorescence spectroscopy. IPCS activity was confirmed for both sets of microsomal material due to the presence of NBD-C_6-IPC **35**. Furthermore, upon the addition of aureobasidin A (AbA) **33**, a potent yeast AUR1 inhibitor, at 5 μM, ablation of activity was observed for *Sc*IPCS but not *Lmj*IPCS. This agrees with previously reported findings,[159] confirming the successful complementation of Δ*AUR1 S. cerevisiae* with the *Leishmania major* IPC synthase.

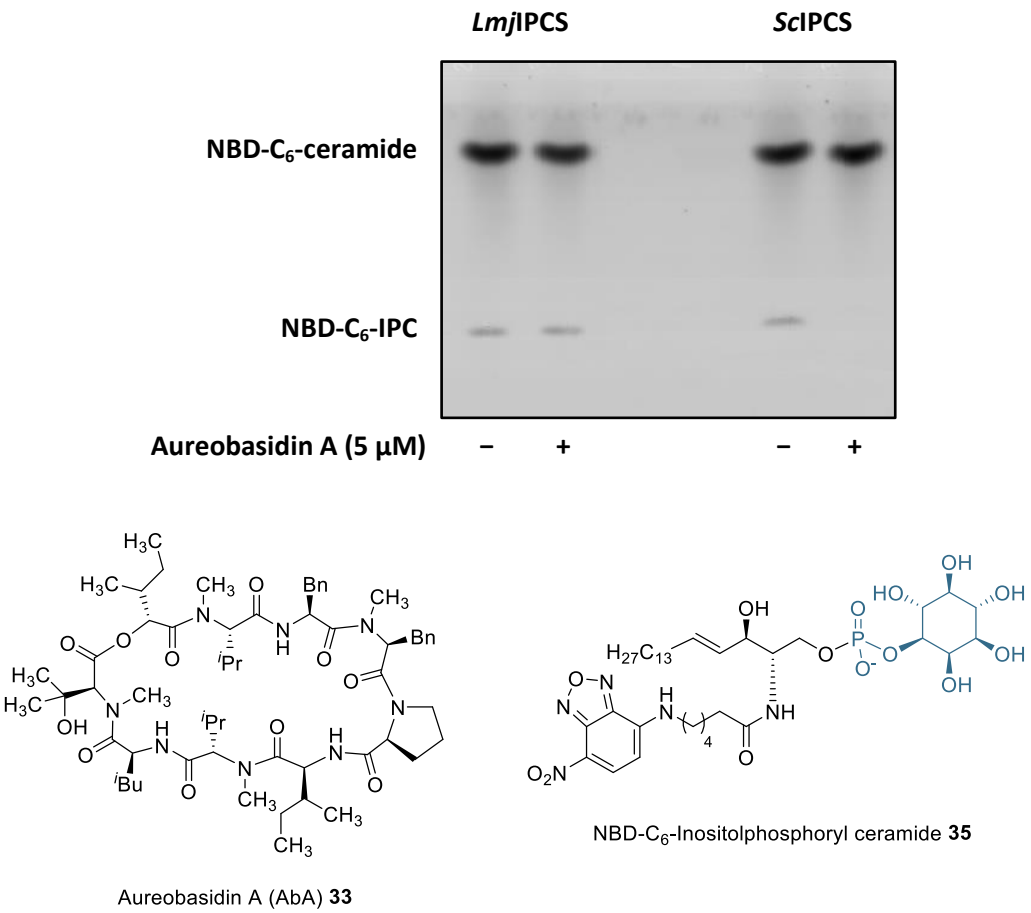


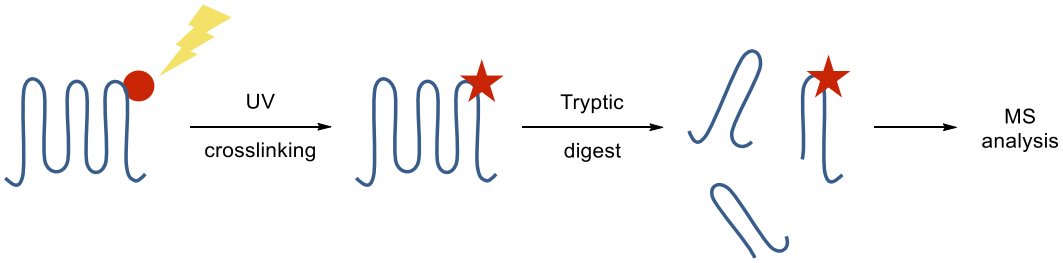
Figure 6–3: Microsomal membranes from Δ*AUR1* pESC–LEU–*LmjIPCS* and Δ*AUR1* pESC–LEU–*ScAUR1 S. cerevisiae* exhibit IPCS activity. Furthermore, AbA 33 inhibits *Sc*IPCS but not *Lmj*IPCS at 5 µM. Experiment conducted by the author.

6.2 Preliminary efforts towards developing photoaffinity probes

6.2.1 Background information

While it is clear that clemastine **41** is an effective inhibitor of the *Leishmania major* IPCS, the mechanism by which this activity is achieved is unknown. Therefore, the precise action of clemastine-like inhibitors requires experimental verification. As X-ray crystallographic methods are not easily applicable to the membrane bound IPC synthase, other approaches must be employed. The use of photoaffinity probes, coupled with mass spectrometry (and MS/MS) analyses, have been reported in the literature for the identification and characterisation of protein targets.[262,263] In this, a protein and photoactive probe are incubated until sufficient binding is achieved. On irradiation, a highly reactive species, such as a nitrene or carbene, is generated which inserts into the protein to provide a cross-linked species (Scheme 6–2).

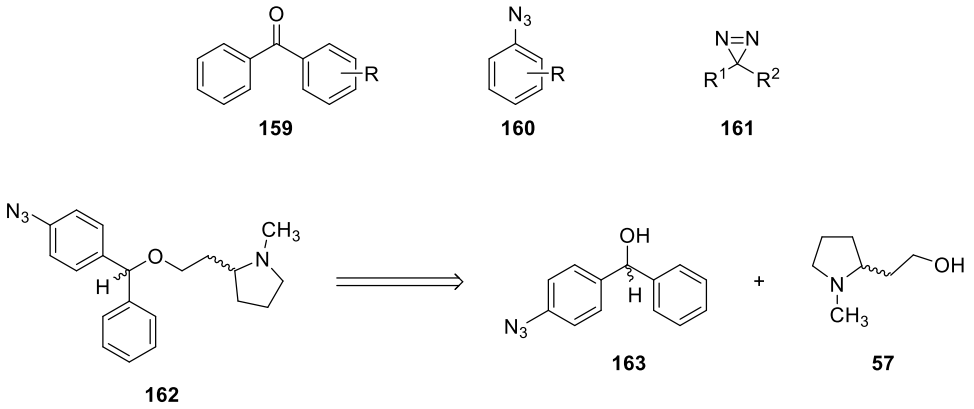
Subsequent proteolytic cleavage (e.g. using trypsin) yields fragments which can then be analysed using mass spectrometry. Comparing the output with that obtained from non-labelled protein enables the sites of probe-binding to be elucidated. Confirmation can be obtained by modifying these residues *via* site-directed mutagenesis to create engineered proteins which are resistant to the probe molecule.[264]



Scheme 6–2: Characterisation of a protein using a photolabelling-mass spectrometry experiment

A suitable probe molecule must fulfil certain criteria, such as chemical stability under enzymatic conditions, short irradiation period (to minimise potential damage to the protein target), a suitable wavelength for irradiation and a low propensity for non-specific labelling (*i.e.* irradiated species have a life span which enables efficient crosslinking).[263,265] Importantly, a synthesised probe must have comparable activity to the inhibitor it is based on. In addition, a photoaffinity probe may also bear other features such as fluorescent moieties, biotin residues or radioisotopic labels, to allow for facile identification and separation of labelled peptides.[262] This method could potentially be used with membrane-bound proteins, such as *Lmj*IPCS, where a crosslinked probe may be used as a purification tag, to remove the enzyme from other membrane components prior to analysis.

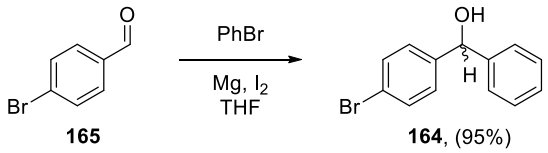
The most commonly used photoreactive groups are benzophenones **159**, aryl azides **160** and diazirines **161** (Scheme 6–3).[265] Using clemastine **41** as a scaffold, it was viewed that introducing one of these functionalities onto an aryl ring would be relatively straightforward. A preliminary synthetic attempt focussed on the construction of aryl azide clemastine analogue **162**, as it was assumed that the azide precursor **163** could be rapidly accessed.



Scheme 6–3: Example photoreactive groups

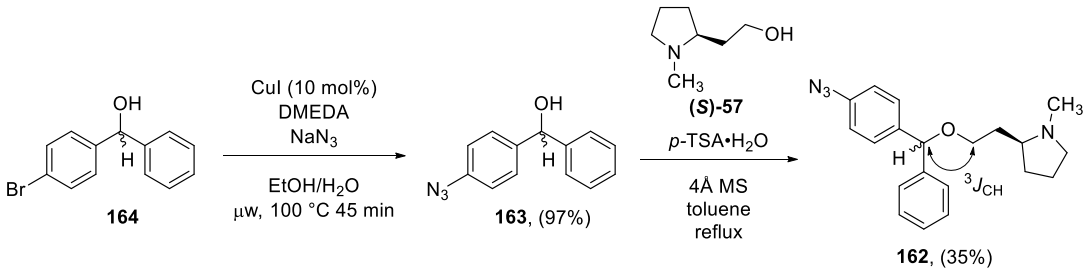
6.2.2 Synthesis of azide analogue 162

Following precedent by Andersen *et al.*, it was viewed that the azide benzhydrol **163** could be accessed from the corresponding aryl bromide **164** (Scheme 6–4).[266] 4-Bromobenzaldehyde **165** was reacted with freshly prepared phenyl Grignard reagent, furnishing the desired bromide precursor **164** in excellent yield, as evidenced by the presence of an IR-active O–H vibration at 3352 cm^{-1}.



Scheme 6–4: Synthesis of *para*-bromo benzhydrol 164

Next, aryl bromide **164** was reacted with sodium azide in the presence of a copper catalyst under microwave heating (Scheme 6–5).[266] Infrared spectroscopic analysis of the resultant crude material revealed a characteristic azide vibration at 2120 cm^{-1}. Without purification, the crude azide **163** was coupled with homoprolinol **(*S*)-57** to afford the desired ether **162**, as confirmed by a 2D NMR HMBC experiment, which revealed coupling, between the benzylic (δ_H = 5.30 ppm) and methylene (δ_H = 3.53 – 3.43 ppm) positions.



Scheme 6–5: Synthesis of azide clemastine analogue 162

6.2.1 *In vitro* testing of azide analogue 162

The azide analogue **162** was tested against the *L. major* IPCS enzyme in a dose-response study (Figure 6–4). Azide **162** exhibited micromolar activity against the enzyme (IC_{50} = 3.66 µM, 95% CI: 3.24 – 4.14 µM), at concentrations comparable to the clemastine analogue **78A** (IC_{50} = 4.45 µM, 95% CI: 4.01 – 4.94 µM).

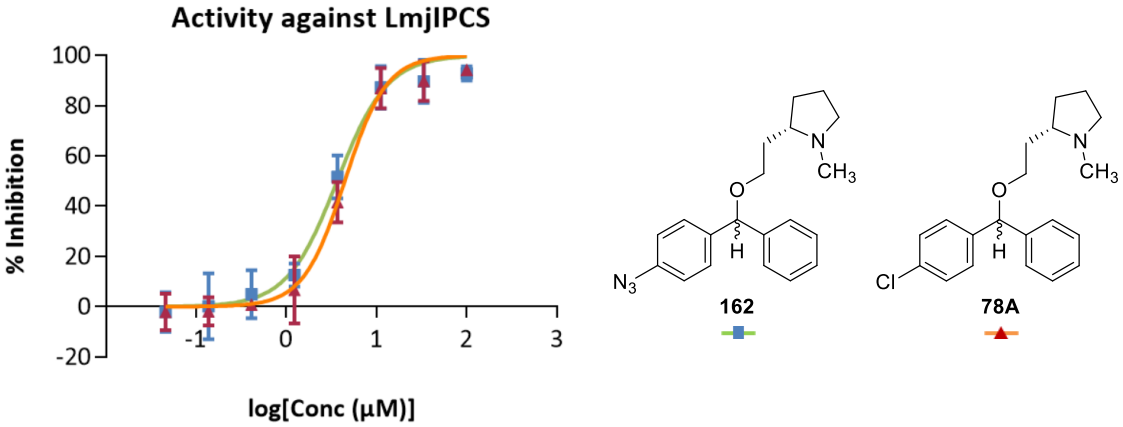


Figure 6–4: Biochemical activity of azide 162 against *Lmj*IPCS. Nor-clemastine 78A is shown for comparison. Error bars portray 95% confidence interval.

Next, an assay against *L. major* promastigotes revealed the azide analogue **162** to have lower antileishmanial activity (ED_{50} = 2.85 µM, 95% CI: 2.28 – 3.56 µM) than nor-clemastine **78A** by a factor of ~2 (ED_{50} = 1.69 µM, 95% CI: 1.23 – 2.32 µM) (Figure 6–5). The contribution of the azide moiety to this modest decrease in activity remains unclear.

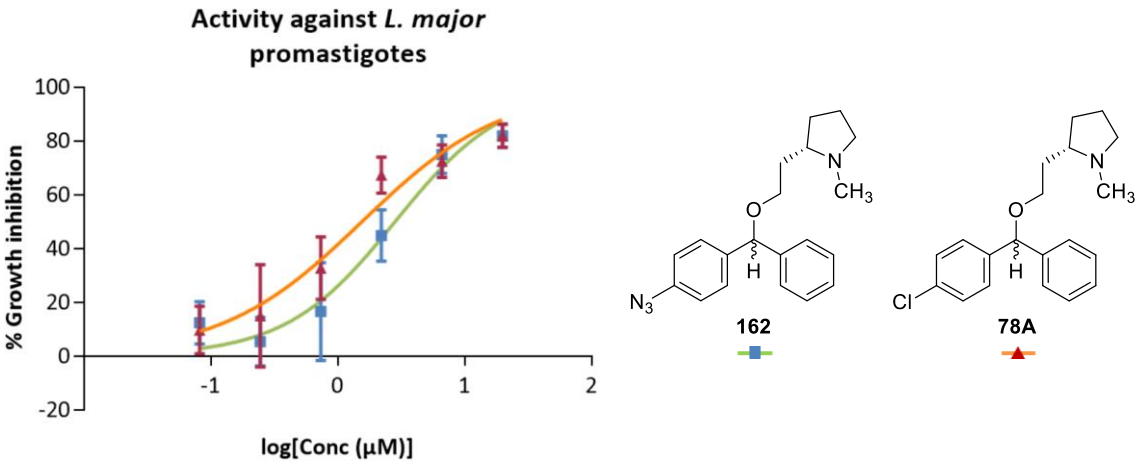


Figure 6–5: Comparison of antileishmanial activity of azide 162 and clemastine analogue 78A. Error bars portray 95% confidence interval.

6.2.2 UV absorption measurement of azide 162

Once the inhibitory effect of azide **162** on *L. major* IPCS activity and promastigote growth was confirmed, the UV absorption profile of the potential photoaffinity probe was measured (Figure 6–6). Upon analysis of the obtained data, it was apparent that the absorption band of interest (235 – 275 nm) was similar to the 'near-UV' region (240 – 300 nm) generally observed in the UV spectra of proteins.[267,268] This is a common drawback of aryl azide probes,[269] but may be circumvented by modifying substituents on the aromatic ring, thereby altering the photochemical properties of the probe.[270] Measurement of the UV absorbance of *Lmj*IPCS would allow for a direct comparison, however the presence of other membrane components would interfere, requiring the deconvolution of any obtained spectra. Furthermore, removal of the membrane lipids would risk unfolding the enzyme, which would furnish an unreliable UV absorption spectrum.[268]

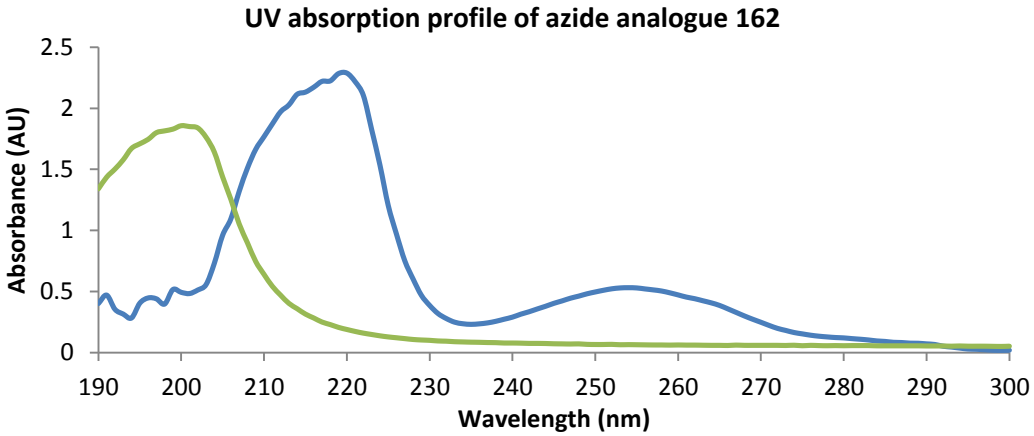


Figure 6–6: UV absorption profile of azide 162 (blue line) at 25 µM in 50 mM phosphate buffer with 600 µM CHAPS. The green line represents blank buffer.

6.2.3 Conclusions

In conclusion, azide **162** was synthesised and found to maintain activity against the *L. major* IPCS enzyme and promastigote parasites, when compared to nor-clemastine **78A**. However, an assessment of its potential as a photoaffinity probe revealed the lack of a suitable irradiation wavelength which would not be liable to interference from biological material.

Future work should focus on the synthesis of substituted aryl azides, which may influence UV absorption (Figure 6–7).[270] Additionally, an exploration of other clemastine derivatives featuring the benzophenone and diazirine photoreactive groups may reveal a promising alternative compound series. Identification of a viable crosslinking moiety may then inform the syntheses of analogues bearing additional functionality, such as purification tags or fluorescent groups.

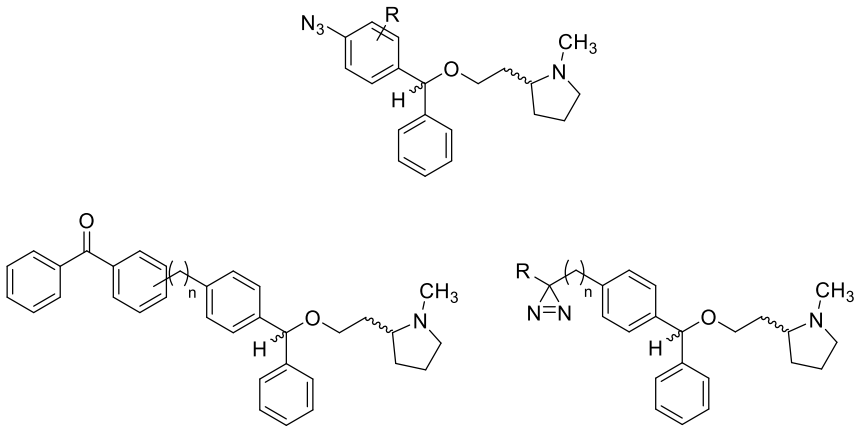


Figure 6–7: Other potential clemastine-based photoaffinity probes

7. Conclusions and Further Work

7.1 Conclusions

This thesis comprises efforts made towards the discovery and development of novel small molecule inhibitors of the *Leishmania major* inositol phosphoryl ceramide synthase (*Lmj*IPCS) enzyme, with a view to elucidating information about the enzyme whilst uncovering potential new therapies for leishmaniasis.

A screening campaign using the 1040 member NINDS compound library revealed clemastine **41** to be an effective inhibitor of *Lmj*IPCS. Importantly, clemastine **41** also displayed antileishmanial activity against the pathologically relevant amastigote form of *Leishmania major*, through its tentative role as an inhibitor of IPC production.

In order to probe the possible interactions between clemastine **41** and *Lmj*IPCS, a range of analogues were prepared, the majority through a facile peptide coupling strategy. The synthesised analogue compounds were screened against the enzyme, revealing the following structure-activity relationships (SARs) (Figure 7–1).

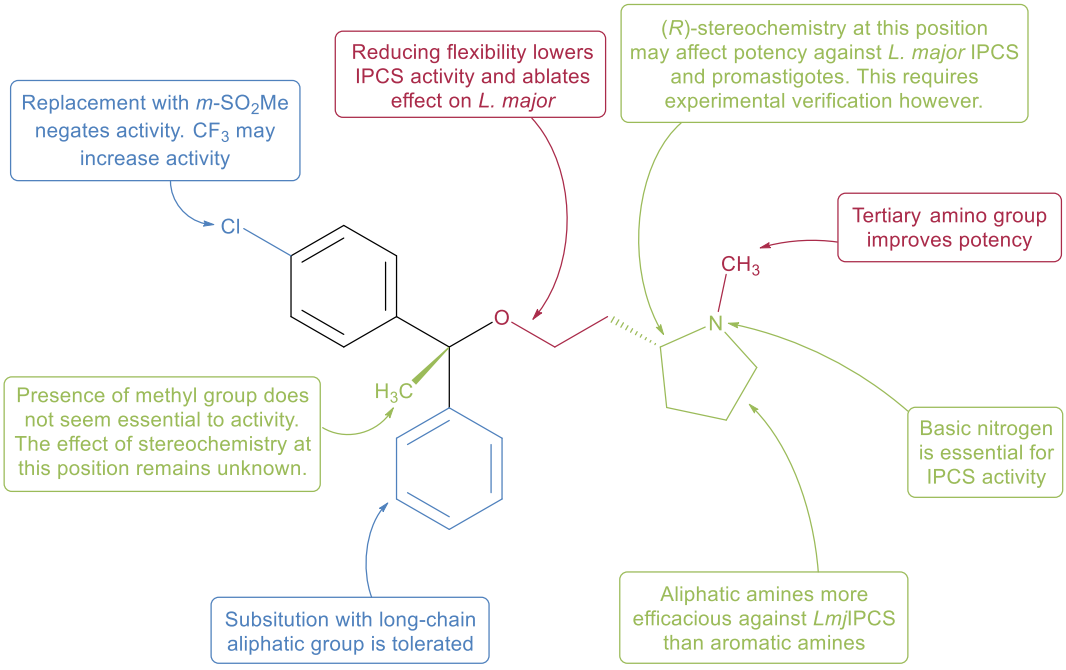


Figure 7–1: A summary of SAR observations

Of the 61 amide-containing analogues screened, indazole **118** was found to be the most effective against *Lmj*IPCS, displaying similar potency to clemastine **41** (Figure 7–2). However, indazole **118** was found to be ineffective against *L. major* promastigote parasites, a drawback

which was not rectified through replacement of the amide moiety with ether functionality (indazole **146**).

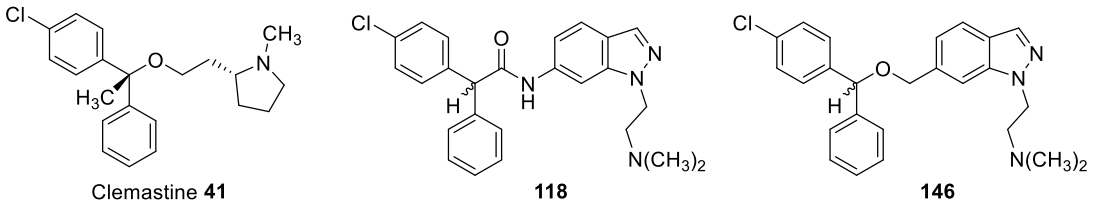


Figure 7–2: Analogues of clemastine 41 bearing an indazole moiety

Another investigation into the possible interactions between clemastine **41** and *Lmj*IPCS focused on the development of a photoaffinity probe. Azide **162** was prepared in a straightforward manner, and exhibited biochemical and antileishmanial activity similar to that of clemastine **41**. However it's measured UV spectrum revealed no suitable target wavelength for activation of the putative probe **162** in the presence of enzymatic material.

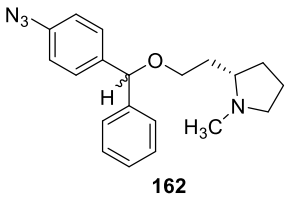


Figure 7–3: Azide analogue 162

7.2 Further work

Although an effective antileishmanial compound based on the clemastine **41** scaffold was not found, there remain a number of avenues of exploration for this compound class. Further efforts may focus on investigating the aryl substituents of the benzhydryl moiety, the position of the nitrogen on the pyrrolidine ring, and the effect of linker chain length on activity.

More fundamentally, an in-depth study on the impact of benzhydryl and pyrrolidinyl stereochemistry on *Lmj*IPCS inhibition may point towards important enzyme-inhibitor interactions. This would require separate synthesis, where necessary, and screening of the four possible diastereomers of clemastine **41**: (*R*,*R*), (*R*,*S*), (*S*,*R*) and (*S*,*S*).

Structural studies of the *Lmj*IPCS enzyme represent another significant area for further investigation. As described in Chapter 6, a continuation of efforts around the generation of photoaffinity probes may focus on the synthesis of analogues bearing substituted aryl azides, benzophenone or diazirine derivatives (Figure 7–4). Once a suitable labelling tool is found,

further work should focus on the development of a robust protocol for isolation of the *Lmj*IPCS enzyme, such that enzyme-probe adducts may be suitably analysed. This may be done through the addition of purification tags to either the probe molecule or the *Lmj*IPCS enzyme itself.

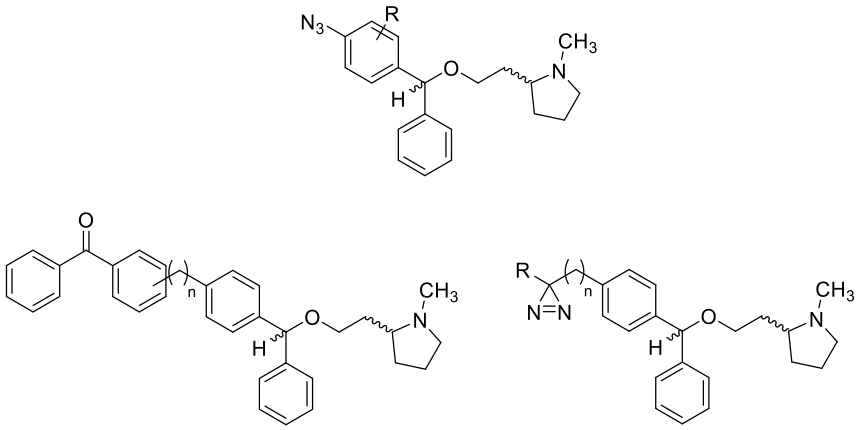


Figure 7–4: Potential clemastine-based photoaffinity probes

Computational studies may also be undertaken to support the above described SAR and photolabelling studies. For example, Neuwald reported an observed relationship in the structures and catalytic mechanisms of membrane phosphatases (e.g. LPPs) and haloperoxidase enzymes, for which resolved crystal structures are known.[271] As the conservation of catalytic residues among LPP enzymes and sphingolipid synthases has also been demonstrated,[212] it may be possible to build a putative 3D model of the catalytic site of *Lmj*IPCS, based on known haloperoxidases. An *in silico* model could shed light on the interaction, if any, of clemastine analogues with the catalytic site, adding a rational design element to further analogue synthesis.

Lastly, the initial screen of the NINDS set revealed chemical scaffolds other than clemastine **41**, which may be used as a starting point for other SAR studies. Examples include amlodipine **39**, flunarizine **45** and suloctidil **52**. However, these compounds provide limited scope in that their activity against intramacrophage *L. major* amastigotes could not be accurately assessed. Therefore, SAR studies based on these compounds may not yield effective antileishmanials.

In the long term, the approaches discussed throughout this thesis may be applied to an investigation of the IPCS enzymes of other *Leishmania* species, such that vital information may be gained concerning the biosynthesis of sphingolipids within these complex organisms.

8. Chemical Experimental Details

8.1 General experimental details

SOLVENTS AND REAGENTS: Solvents and commercially available reagents were dried and purified as required, using standard procedures. DCM, DMF, DMSO, ether (diethyl ether), hexanes, MeCN, MeOH and THF were obtained dry from Inert Techonolgy Inc. SPS (solvent purification systems) and stored under argon. *N*,*N*'-Dimethylethylenediamine (DMEDA) and triethylamine were distilled from, and stored over, KOH. Petrol refers to the fraction of petroleum ether boiling in the range 40 – 60 °C. All reactions were performed in round bottomed flasks, with stirring, under an atmosphere of argon unless otherwise stated. Butyllithium refers to n-butyllithium.

CHROMATOGRAPHY: Thin layer chromatography (TLC) was carried out using Merck aluminium or glass backed precoated plates (silica gel 60 Å F_{254}). Compounds were visualised under UV radiation (λ_{max} = 254 nm and 365 nm) and then stained, using the appropriate reagent, and heated. Retention factors (R_f) are reported along with the solvent system used in parenthesis. Purification *via* flash column chromatography was conducted manually on silica gel (40 – 60 µm, 60 Å) or automatically using a CombiFlash® system with normal-phase and C-18 reversed-phase silica gel columns (purchased from Teledyne Isco Inc.).

NUCLEAR MAGNETIC RESONANCE: ^{1}H and ^{13}C NMR spectra were recorded on Varian VNMRS 700 (^{1}H at 700 MHz, ^{13}C at 176 MHz), Varian VNMRS 600 (^{1}H at 600 MHz, ^{13}C at 151 MHz), Inova 500 (^{1}H at 500 MHz) and Bruker Avance-400 (^{1}H at 400 MHz, ^{13}C at 101 MHz) instruments at room temperature, unless otherwise stated. ^{1}H-coupled ^{19}F NMR spectra were recorded on Bruker Avance-400 and Varian Mercury-400 instruments at a frequency of 376 MHz. Chemical shifts (δ_H or δ_C) are reported in parts per million (ppm) of tetramethylsilane using residual solvent as an internal standard. Coupling constants (*J*) are quoted to the nearest 0.5 Hertz (Hz). The multiplicity is indicated by singlet (s), doublet (d), triplet (t), quartet (q), quintet (quin), heptet (hept), multiplet (m), broad (br) or a combination thereof. Al and Ar refer to the respective alkyl and aryl resonances which could not be accurately assigned. In the case of rotameric compounds, proton and carbon resonances of the major and minor rotamers are assigned as A and B respectively, with their relative integration values reported. Diastereomeric resonances are labelled arbitrarily as 1 and 2, where elucidation of the separate isomers is possible.

INFRARED SPECTROSCOPY: Infrared (IR) spectra were recorded using a Perkin-Elmer Paragon 1000 FT-IR with Diamond ATR module. Absorption maxima (ν_{max}) are reported in wavenumbers (cm^{-1}) and are described as strong (s), medium (m), weak (w) or broad (br) or a combination thereof.

MASS SPECTROMETRY: Low resolution mass spectra (LRMS) were recorded *via* electron ionisation (EI), using Thermo Trace and Shimadzu gas chromatography (GC) instruments, or electrospray ionisation (ESI), using a Waters TQD spectrometer equipped with an Acquity UPLC. High resolution mass spectra (HRMS) were obtained using Waters LCT Premier XE or QTOF Premier instruments (ESI). Mass to charge ratios (m/z) are reported in Daltons with the corresponding fragment ion, where known.

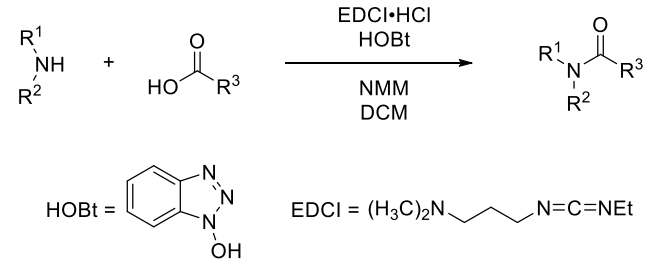
OPTICAL ROTATION: Measurements were performed on a JASCO P-1020 polarimeter, and are stated alongside temperature, solvent and concentration, in $mgmL^{-1}$ with corresponding literature values.

MELTING POINTS: Melting points were determined using Fisher Scientific™ IA9000 series melting point apparatus and are uncorrected. Decomp. refers to material that decomposed upon heating.

MICROWAVE MEDIATED REACTIONS: Reactions under microwave irradiation conditions were performed using monomodal Emrys™ Optimizer from Personal Chemistry, in sealed microwave process vials. The time reported reflects the time that the reaction mixture was kept at designated temperature (fixed hold time) by varying irradiation power.

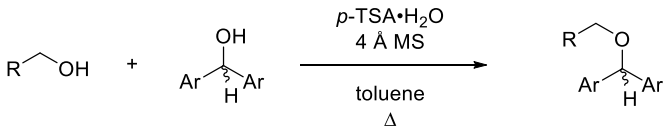
8.2 Experimental procedures

General procedure A: Amide bond formation



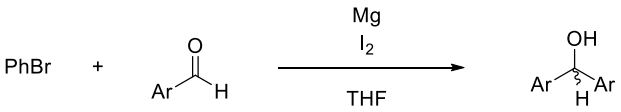
Procedure:[246] To a mixture of amine (1 eq.), *N*-methylmorpholine (NMM) (1.1 eq.) and EDCI•HCl (1.1 eq.) in DCM (3 mL.mmol^{-1}) at 0 °C was added acid (1 eq.) and HOBt (1.1 eq.) in DCM (3 mL.mmol^{-1}). DMF was added as required to aid solvation. After being stirred at RT overnight, the reaction mixture was quenched with H_2O (4 mL.mmol^{-1}) and passed through a phase separator. The resultant solution was concentrated under reduced vacuum and purified *via* reversed-phase flash column chromatography.

General procedure B: Condensation of two alcohols



Procedure:[233] A flask fitted with a water condenser was charged with primary alcohol (1 eq.), benzhydryl alcohol (1.1 eq.), *p*-toluenesulfonic acid monohydrate (1.1 eq.), toluene (4 mL.mmol^{-1}) and 4 Å molecular sieves (100 mg.mmol^{-1}). The reaction mixture was heated to reflux for 2 h before being cooled and quenched by the addition of 1 M $NaOH_{(aq.)}$ (4 mL.mmol^{-1}). The mixture was extracted with EtOAc and the organic extracts washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. Purification of the resultant crude material was performed on SiO_2 column.

General procedure C: Preparation of benzhydryl alcohols



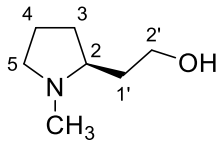
Procedure:[272] To a 2-necked round bottomed flask equipped with a reflux condenser and a pressure equalising addition funnel was added Mg turnings (1.6 eq.), THF (0.1 mL.mmol^{-1}) and a small portion of bromobenzene (0.16 eq.). The reaction was initiated by addition of a crystal of I_2 and gentle warming until reflux temperature was reached. This reflux was maintained by slow addition of bromobenzene (1.5 eq.) in THF (0.5 mL.mmol^{-1}). After addition was complete, the reaction mixture was heated to reflux for 1 h. The freshly prepared Grignard reagent was then cooled in an ice bath before benzaldehyde (1 eq.) in THF (1 mL.mmol^{-1}) was added dropwise. The reaction mixture was warmed to RT and reacted for 4 h, after which the solution was poured onto ice-cold saturated $NH_4Cl_{(aq.)}$. THF was removed under reduced pressure and the residue extracted with EtOAc. The combined organic extracts were washed with brine and dried over $MgSO_4$ prior to concentration *in vacuo*. The crude material was purified by silica column chromatography.

General procedure D: Fieser's method for the workup of $LiAlH_4$ reactions

Procedure:[273] A reaction containing X g of lithium aluminum hydride was first cooled to 0 °C and diluted with ether. Then, cold H_2O (X mL) was slowly added, followed by 3 M $NaOH_{(aq.)}$ (X mL) and H_2O (3X mL). The quenched mixture was warmed to RT and stirred for 15 min, after which $MgSO_4$ was added and the mixture stirred for an additional 5 min. The combined lithium and magnesium salts were then removed by filtration, before removal of volatiles under reduced pressure afforded the crude product.

8.3 Compound characterisation

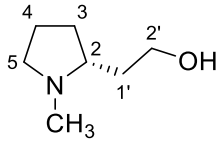
(2*S*)-2-(2′-Hydroxyethyl)-1-methylpyrrolidine[222] (*S*)-57



Procedure:[222] To a 0 °C solution of methyl ester **(*S*)-70** (671 mg, 2.42 mmol) in THF (12 mL) was slowly added $LiAlH_4$ (2.4 M solution in THF, 3.00 mL, 7.26 mmol), before being allowed to warm to RT. After 3 h the mixture was cooled once more in an ice bath and quenched according to Fieser's method (general procedure D).[273] The combined organic filtrates were concentrated *in vacuo* and purified by SiO_2 column (0% → 10% MeOH in $CHCl_3$ with 1% NEt_3) to afford the title compound (309 mg, 99%) as a thick clear colourless oil.

No optical rotation measurement was obtained. R_f 0.18 (50% EtOAc in hexanes); ν_{max} (ATR) 3288 (br), 2941 (m), 2840 (m), 2792 (m), 1455 (m), 1210 (w), 1056 (s), 1016 (m) cm^{-1}; δ_H (600 MHz, $CDCl_3$) 5.18 – 5.04 (1H, s, O***H***), 3.92 (1H, td, *J* = 10.5, 3.5 Hz, 2′-***H***H′), 3.66 – 3.59 (1H, m, 2′-H***H***′), 3.06 – 3.00 (1H, m, 5-***H***H′), 2.57 – 2.50 (1H, m, 2-***H***), 2.33 (3H, s, NC$\boldsymbol{H}_3$), 2.18 – 2.10 (1H, m, 5-H***H***′), 1.96 – 1.82 (2H, m, 3-***H***H′, 1′-***H***H′), 1.82 – 1.66 (3H, m, 3-H***H***′, 4-$\boldsymbol{H}_2$), 1.53 – 1.46 (1H, m, 1′-H***H***′); δ_C (151 MHz, $CDCl_3$) 65.2 (***C***-2), 60.3 (***C***-2′), 57.0 (***C***-5), 40.9 (N***C***H_3), 31.8 (***C***-1′), 28.5 (***C***-3), 23.1 (***C***-4); *m/z* (LCMS, ESI^+) 130 (MH^+).

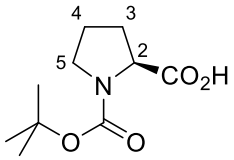
(2*R*)-2-(2′-Hydroxyethyl)-1-methylpyrrolidine[274] (*R*)-57



Procedure:[222] Methyl ester **(*R*)-70** (405 mg, 1.46 mmol) was reduced according to the conditions for the synthesis of alcohol **(*S*)-57** to afford the title compound (122 mg, 65%) as a thick clear colourless oil.

No optical rotation measurement was obtained. R_f 0.18 (50% EtOAc in hexanes); ν_{max} (ATR) 3318 (br), 2945 (m), 2876 (m), 2848 (m), 2786 (m), 1455 (m), 1058 (m), 1018 (m) cm^{-1}; δ_H (600 MHz, $CDCl_3$) 3.98 (1H, ddd, *J* = 11.0, 10.5, 3.0 Hz, 2′-***H***H′), 3.69 – 3.65 (1H, m, 2′-H***H***′), 3.04 (1H, ddd, *J* = 9.5, 7.0, 2.0 Hz, 5-***H***H′), 2.59 – 2.54 (1H, m, 2-***H***), 2.35 (3H, s, NC$\boldsymbol{H}_3$), 2.15 (1H, td, *J* = 9.5, 7.0 Hz, 5-H***H***′), 1.97 (1H, ddt, *J* = 15.0, 10.5, 4.5 Hz, 1′-***H***H′), 1.92 – 1.85 (1H, m, 3-***H***H′), 1.82 – 1.71 (3H, m, 3-H***H***′, 4-$\boldsymbol{H}_2$), 1.48 (1H, dtd, *J* = 15.0, 3.5, 3.0 Hz, 1′-H***H***′); δ_C (151 MHz, $CDCl_3$) 65.3 (***C***-2), 60.4 (***C***-2′), 57.1 (***C***-5), 41.0 (N***C***H_3), 31.5 (***C***-1′), 28.4 (***C***-3), 23.3 (***C***-4); m/z (LCMS, ESI^+) 130 (MH^+).

(2*S*)-(*N*-*tert*-Butoxycarbonyl)pyrrolidine-2-carboxylic acid[275] (*S*)-58

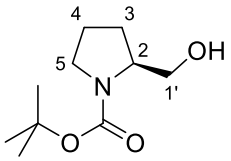


Procedure:[275] To a stirring biphasic mixture of saturated $NaHCO_{3(aq.)}$ (50 mL) and THF (17 mL) was added L-proline (4.00 g, 34.74 mmol). Once the proline was fully dissolved, the mixture was cooled in an ice bath before Boc_2O (97%, 9.05 mL, 38.22 mmol) was added dropwise. The reaction mixture was warmed to RT and stirred for 16 h. Following this, THF was removed under reduced pressure and the remaining mixture cooled to 0 °C before being acidified to pH = 2 using 1M $HCl_{(aq.)}$ solution. This was extracted with EtOAc (3 × 100 mL), the combined organic extracts being washed with brine (70 mL) and dried over $MgSO_4$, before being concentrated under reduced pressure and dried under high vacuum to afford the title compound (7.39 g, 99%) as a white solid.

Melting point data were not obtained. $[\alpha]^{20}_D$ (c = 1.00 g/100 mL, $CHCl_3$) −90.6° (lit.:[275] $[\alpha]^{20}_D$ (c = 1.38 g/100 mL, $CHCl_3$) −80.0°); ν_{max} (ATR) 3070 (br), 2971 (m), 2876 (w), 1736 (m), 1689 (s), 1655 (s), 1391 (s), 1365 (m), 1241 (m), 1162 (s), 1128 (s) cm^{-1}; δ_H (600 MHz, $CDCl_3$, mixture of rotamers) 4.31 – 4.27 (0.6H, m, 2-***H*** rotamer A), 4.20 – 4.16 (0.4H, m, 2-***H*** rotamer B), 3.52 – 3.46 (0.4H, m, 5-***H***H′ rotamer B), 3.42 – 3.36 (1H, m, 5-H***H***′), 3.32 – 3.26 (0.6H, m, 5-***H***H′ rotamer A), 2.28 – 2.17 (1.2H, m, 3-***H***H′ rotamer A, 4-***H***H′ rotamer A), 2.04 – 1.96 (0.8H, m,

3-***H***H′ rotamer B, 4-***H***H′ rotamer B), 1.92 – 1.79 (2H, m, 3-H***H***′, 4-H***H***′), 1.42 (5.4H, s, C(C***H***$_3$)$_3$ rotamer A), 1.36 (3.6H, s, C(C***H***$_3$)$_3$ rotamer B); δ_C (151 MHz, $CDCl_3$, mixture of rotamers) 178.7 (***C***O_2H rotamer B), 174.7 (***C***O_2H rotamer A), 156.6 (***C***=O rotamer A), 153.8 (***C***=O rotamer B), 81.5 (***C***$(CH_3)_3$ rotamer A), 80.3 (***C***$(CH_3)_3$ rotamer B), 59.2 (***C***-2 rotamer A), 58.9 (***C***-2 rotamer B), 47.0 (***C***-5 rotamer A), 46.3 (***C***-5 rotamer B), 30.8 (***C***-3 rotamer B), 28.4 (C(***C***$H_3)_3$), 28.3 (***C***-3 rotamer A), 24.5 (***C***-4 rotamer A), 23.6 (***C***-4 rotamer B); *m/z* (LCMS, ESI^-) 214 (M − H).

(2*S*)-(*N*-*tert*-Butoxycarbonyl)-2-(hydroxymethyl)pyrrolidine[276] (*S*)-59

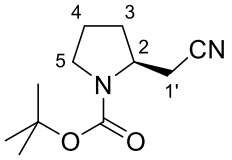


Procedure :[276] A round bottomed flask equipped with a reflux condenser was charged with *N*-Boc-L-proline **(*S*)-58** (3.42 g, 15.88 mmol) and THF (80 mL). The mixture was cooled in an ice bath before dropwise addition of a solution of BH_3·SMe_2 (1.70 mL, 17.93 mmol) in THF (9 mL). The reaction mixture was heated to reflux for 1 h, after which complete conversion of starting material was observed by TLC. After cooling to 0 °C, the reaction was quenched with the addition of cold H_2O (5 mL) and THF was removed from the crude mixture *in vacuo*. The residual material was diluted with H_2O (100 mL) and extracted with DCM (3 × 30 mL). The combined organic extracts were washed with brine (60 mL) and dried over $MgSO_4$ before being concentrated under reduced pressure to furnish the title compound (3.14 g, 98%) as a clear viscous oil.

R_f 0.55 (4:1:1, petrol : EtOAc : EtOH); $[\alpha]^{21}_D$ (c = 1.00 g/100 mL, $CHCl_3$) −29.5° (lit.:[223] $[\alpha]^{25}_D$ (c = 1.20 g/100 mL, $CHCl_3$) −39.8°); ν_{max} (ATR) 3403 (br), 2976 (m), 2928 (w), 2881 (m), 1663 (s), 1391 (s), 1365 (m), 1244 (w), 1162 (s), 1106 (m) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 4.73 (1H, d, *J* = 6.0 Hz, O***H***), 3.96 – 3.89 (1H, m, 2-***H***), 3.63 – 3.58 (1H, m, 1′-***H***H′), 3.58 – 3.53 (1H, m, 1′-H***H***′), 3.46 – 3.38 (1H, m, 5-***H***H′), 3.31 – 3.25 (1H, m, 5-H***H***′), 2.02 – 1.93 (1H, m, 3-***H***H′), 1.86 – 1.78 (1H, m, 4-***H***H′), 1.77 – 1.71 (1H, m, 4-H***H***′), 1.55 – 1.48 (1H, m, 3-H***H***′), 1.44 (9H, s,

C(C***H***$_3$)$_3$); δ_C (176 MHz, $CDCl_3$) 157.1 (***C***=O), 80.2 (***C***(CH$_3$)$_3$), 67.6 (***C***-1'), 60.1 (***C***-2), 47.5 (***C***-5), 28.7 (***C***-3), 28.4 (C(***C***H$_3$)$_3$), 24.0 (***C***-4); *m/z* (LCMS, ESI$^+$) 202 (MH^+), 224 (MNa^+).

(2*S*)-(*N*-*tert*-Butoxycarbonyl)-2-(cyanomethyl)pyrrolidine[277] (*S*)-61



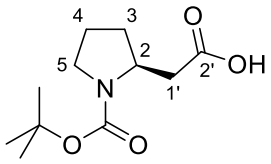
Procedure:[224] To a 0 °C solution of alcohol **(*S*)-59** (819 mg, 4.07 mmol) in DCM (8 mL) was added NEt_3 (1.26 mL, 8.95 mmol) then *p*-toluenesulfonyl chloride (98%, 1.16 g, 6.10 mmol). The reaction mixture was warmed to RT and stirred for 15 h, after which time TLC analysis showed consumption of starting material. The reaction mixture was diluted with H_2O (10 mL) and extracted with DCM (3 × 10 mL). The combined organic extracts were washed with saturated $NH_4Cl_{(aq.)}$ (20 mL), brine (20 mL) and saturated $NaHCO_{3(aq.)}$ (20 mL) before being dried over $MgSO_4$ and concentrated *in vacuo*. The crude material was briefly purified by column chromatography (5% → 20% EtOAc in petrol) to give tosylate **(*S*)-60** (1.28 g, 88%) as an unstable thick clear oil which could not be characterised and was taken immediately forward to the next step.

To a solution of tosylate **(*S*)-60** (710 mg, 2.00 mmol) in dry DMSO (2 mL) was added NaCN (277 mg, 5.65 mmol) and the reaction mixture stirred at 90 °C for 4 h. After cooling to RT, the mixture was diluted with H_2O (20 mL) and the aqueous layer extracted with ether (4 × 15 mL). The combined organic extracts were then washed with brine (20 mL), dried over $MgSO_4$ and concentrated *in vacuo* before being passed through a SiO_2 plug (30% EtOAc in hexanes) to afford the title compound (233 mg, 55%) as a pale orange oil.

No optical rotation measurement was obtained. R_f 0.27 (30% EtOAc in hexanes); ν_{max} (ATR) 2971 (w), 2885 (w), 2242 (w), 1685 (s), 1387 (s), 1158 (m), 1115 (w) cm^{-1}; δ_H (700 MHz, $CDCl_3$, mixture of rotamers) 4.02 – 3.96 (1H, m, 2-***H***), 3.48 – 3.43 (0.4H, m, 5-***H***H' rotamer B), 3.43 – 3.35 (1.6H, m, 5-***H***$_2$ rotamer A, 5-H***H***' rotamer B), 2.86 – 2.79 (0.6H, m, 1'-***H***H' rotamer A), 2.73 – 2.64 (1H, m, 1'-H***H***' rotamer A, 1'-***H***H' rotamer B), 2.58 – 2.52 (0.4H, m, 1'-H***H***' rotamer B), 2.19 – 2.11 (1H, m, 3-***H***H'), 2.04 – 1.93 (1H, m, 4-***H***H'), 1.93 – 1.88 (1H, m, 3-H***H***'), 1.88 – 1.81

(1H, m, 4-H***H***'), 1.49 – 1.46 (3.6H, s, C(C***H***$_3$)$_3$ rotamer B), 1.46 – 1.44 (5.4H, s, C(C***H***$_3$)$_3$ rotamer A); δ_C (176 MHz, $CDCl_3$, mixture of rotamers) 154.5 (***C***=O rotamer A), 153.9 (***C***=O rotamer B), 118.0 (***C***N rotamer A), 117.7 (***C***N rotamer B), 80.4 (***C***$(CH_3)_3$ rotamer B), 80.0 (***C***$(CH_3)_3$ rotamer A), 53.7 (***C***-2), 47.1 (***C***-5 rotamer A), 46.7 (***C***-5 rotamer B), 30.4 (***C***-3), 28.4 (C(***C***$H_3)_3$), 23.7 (***C***-4 rotamer A), 23.1 (***C***-4 rotamer B), 22.8 (***C***-1' rotamer B), 22.3 (***C***-1' rotamer A); *m/z* (LCMS, ESI^+) 211 (MH^+), 233 (MNa^+).

(2*S*)-(*N*-*tert*-Butoxycarbonyl)-2-(ethyl-2'-carboxylic acid)pyrrolidine[278] **(*S*)-62**

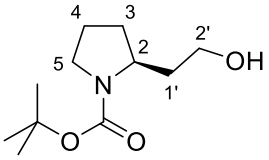


Procedure:[225] To a round bottomed flask equipped with a reflux condenser was added nitrile **(*S*)-61** (250 mg, 1.19 mmol), H_2O (3.6 mL) and THF (2 mL). To this biphasic mixture was added Na_2O_2 (185 mg, 2.38 mmol) slowly and the reaction heated to 70 °C until TLC analysis revealed consumption of starting material. The reaction was heated for a further 2 h before being cooled to 0 °C and acidified to pH = 2 with 1 M $HCl_{(aq.)}$. THF was removed under reduced pressure and the resultant liquid extracted with EtOAc (3 × 10 mL). The combined organic extracts were washed with brine (15 mL) and dried over $MgSO_4$ before being concentrated *in vacuo* to furnish the title compound (205 mg, 75%) as a light yellow solid.

No optical rotation measurement was obtained. Melting point data were not obtained. ν_{max} (ATR) 2976 (w), 2928 (w), 2876 (w), 1719 (m), 1689 (s), 1391 (s), 1162 (s) cm^{-1}; δ_H (700 MHz, $CDCl_3$, mixture of rotamers) 4.18 – 4.06 (1H, m, 2-***H***), 3.40 – 3.27 (2H, m, 5-***H***$_2$), 3.00 – 2.89 (0.5H, m, 1'-***H***H' rotamer A), 2.89 – 2.79 (0.5H, m, 1'-***H***H' rotamer B), 2.39 – 2.29 (1H, m, 1'-H***H*** rotamer A, 1'-H***H*** rotamer B), 2.07 (1H, dq, *J* = 12.5, 8.0 Hz, 3-***H***H'), 1.89 – 1.79 (2H, m, 4-***H***$_2$), 1.79 – 1.71 (1H, m, 3-H***H***'), 1.44 (9H, s, C(C***H***$_3)_3$); δ_C (176 MHz, $CDCl_3$, mixture of rotamers) 176.8 (***C***-2' rotamer A), 176.1 (***C***-2' rotamer B), 154.8 (***C***=O rotamer A), 154.3 (***C***=O rotamer B), 78.9 (***C***$(CH_3)_3$), 53.9 (***C***-2), 46.6 (***C***-5 rotamer A), 46.2 (***C***-5 rotamer B), 39.3 (***C***-1'),

31.3 (***C***-3 rotamer A), 31.2 (***C***-3 rotamer B), 28.4 (C(***C***H_3)$_3$), 23.5 (***C***-4 rotamer A), 22.8 (***C***-4 rotamer B); *m/z* (LCMS, ESI^-) 228 ($M - H^+$).

(2*S*)-(*N*-*tert*-Butoxycarbonyl)-2-(2'-hydroxyethyl)pyrrolidine[278] (*S*)-63



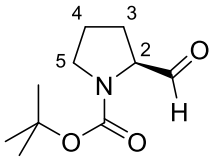
Procedure A:[276] : A round bottomed flask equipped with a reflux condenser was charged with homoproline **(*S*)-62** (184 mg, 0.8 mmol) and THF (4 mL). The mixture was cooled in an ice bath before dropwise addition of a solution of BH_3·SMe_2 (85 µL, 0.90 mmol) in THF (0.5 mL). The reaction mixture was heated to reflux for 1 h, after which complete conversion of starting material was observed by TLC. After cooling to 0 °C, the reaction was quenched with the addition of cold H_2O (1 mL) and THF was removed from the crude mixture under reduced pressure. The residual material was diluted with H_2O (5 mL) and extracted with DCM (3 × 10 mL). The combined organic extracts were washed with brine (20 mL) and dried over $MgSO_4$ before being concentrated *in vacuo* to furnish the enantiomerically pure title compound (162 mg, 94%) as a clear, colourless viscous oil.

Procedure B:[223] To a 0 °C solution of alkene **65** (178 mg, 0.90 mmol) in THF (2.3 mL) was added BH_3·SMe_2 (0.26 mL, 2.71 mmol) and the reaction mixture stirred at RT for 3 h. The reaction was cooled in an ice bath then quenched by the addition of 3 M $NaOH_{(aq.)}$ (0.73 mL) and 27.5% v/v $H_2O_{2(aq.)}$ (0.16 mL), with stirring at RT for a further hour. THF was then removed under reduced pressure and the residue diluted with H_2O (3 mL) and extracted with EtOAc (3 × 5 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated *in vacuo* to provide an inseparable mixture of the racemic title compound (104 mg, 53%) and by-product alcohol **66** (41 mg, 21%) as a clear, colourless thick oil. The ratio of the two products was judged by ^{1}H NMR spectrum of the crude material.

Procedure C:[230] A modified *Method B* was conducted in which cyclohexene (1.05 mL, 10.41 mmol) was added dropwise to a solution of BH_3·SMe_2 (0.49 mL, 5.21 mmol) in THF (3.6 mL) in an ice bath. The reaction mixture was stirred at RT for 45 min before being cooled to 0 °C prior to slow addition of a solution of alkene **65** (685 mg, 3.47 mmol) in THF (4 mL). The reaction was quenched and extracted according to *Method B* to furnish the racemic title compound exclusively (293 mg, 39%) as a clear, colourless viscous oil.

R_f 0.20 (30% EtOAc in petrol); $[\alpha]^{28}_D$ (c = 1.00 g/100 mL, $CHCl_3$) −28.3° (lit.:[223] $[\alpha]^{25}_D$ (c = 1.43 g/100 mL, $CHCl_3$) −10.7°); ν_{max} (ATR) 3429 (br), 2971 (m), 2924 (m), 2871 (m), 1668 (s), 1395 (s), 1365 (w), 1249 (w), 1167 (s), 1102 (w) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 4.40 – 4.35 (1H, m, O***H***), 4.17 – 4.12 (1H, m, 2-***H***), 3.62 – 3.57 (1H, m, 2′-***H***H′), 3.57 – 3.51 (1H, m, 2′-H***H***′), 3.34 – 3.27 (2H, m, 5-$\boldsymbol{H}_2$), 2.00 – 1.94 (1H, m, 3-***H***H′), 1.90 – 1.84 (2H, m, 4-$\boldsymbol{H}_2$), 1.71 – 1.65 (1H, m, 1′-***H***H′), 1.62 – 1.57 (1H, m, 3-H***H***′), 1.45 (9H, s, C($C\boldsymbol{H}_3$)$_3$), 1.47 – 1.40 (1H, m, 1′-H***H***′); δ_C (176 MHz, $CDCl_3$) 156.5 (***C***=O), 79.8 (***C***(CH_3)$_3$), 59.1 (***C***-2′), 53.5 (***C***-2), 46.4 (***C***-5), 38.4 (***C***-1′), 31.2 (***C***-3), 28.4 (C(***C***H_3)$_3$), 23.5 (***C***-4); *m/z* (LCMS, ESI^+) 216 (MH^+), 238 (MNa^+).

(2*S*)-(*N*-*tert*-Butoxycarbonyl)-2-formylpyrrolidine[249] (*S*)-64

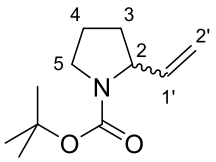


Procedure A:[228] To a water bath cooled solution of prolinol **(*S*)-59** (371 mg, 1.84 mmol) in DCM (9 mL) was added pyridinium chlorochromate (600 mg, 2.78 mmol), 4 Å molecular sieves (1.30 g) and acetic acid (0.16 mL, 2.80 mmol). The reaction mixture was stirred at RT for 2 h followed by addition of celite® (920 mg) and ether (18 mL). The suspension was then filtered over a celite® plug and concentrated under reduced pressure. The residue was passed through a plug of SiO_2 using ether (20 mL) as an eluent. Following removal of solvent under reduced pressure, the crude title compound was obtained (271 mg, 74%) as a yellow oil that was used in the next step without further purification.

Procedure B:[227] To a solution of prolinol **(*S*)-59** (1.70 g, 8.44 mmol) in DMSO (20 mL) was added NEt_3 (3.90 mL, 27.87 mmol), immediately followed by SO_3·pyridine (8.46 g, 53.17 mmol) in DMSO (20 mL). The resulting yellow-orange reaction mixture was stirred at RT until TLC analysis revealed complete conversion of starting material. The reaction was quenched with saturated $NH_4Cl_{(aq.)}$ (20 mL) and extracted with ether (6 × 20 mL). The combined organic extracts were washed with saturated $NH_4Cl_{(aq.)}$ (30 mL), brine (30 mL) then saturated $NaHCO_{3(aq.)}$ (30 mL), before being dried over $MgSO_4$ and concentrated *in vacuo* to provide the crude title compound (1.44 g, 86%) as a pale yellow oil that was used without purification.

No optical rotation measurement was obtained. R_f 0.17 (19:1:1, petrol : EtOAc : EtOH); ν_{max} (ATR) 2971 (m), 2928 (m), 2881 (w), 1784 (w), 1732 (m), 1693 (s), 1391 (s), 1365 (m), 1309 (w), 1249 (w), 1158 (s), 1115 (m) cm^{-1}; δ_H (700 MHz, $CDCl_3$, mixture of rotamers) 9.55 (0.4H, br s, C***H***O rotamer B), 9.45 (0.6H, br s, C***H***O rotamer A), 4.21 – 4.18 (0.4H, m, 2-***H*** rotamer B), 4.06 – 4.01 (0.6H, m, 2-***H*** rotamer A), 3.57 – 3.53 (0.6H, m, 5-***H***H' rotamer A), 3.50 – 3.41 (1.4H, m, 5-H***H***' rotamer A, 5-$\boldsymbol{H}_2$ rotamer B), 2.14 – 2.10 (0.6H, m, 3-***H***H' rotamer A), 2.00 – 1.92 (1.4H, m, 3-H***H***' rotamer A, 3-$\boldsymbol{H}_2$ rotamer B), 1.89 – 1.85 (2H, m, 4-$\boldsymbol{H}_2$), 1.47 (3.6H, s, C(C$\boldsymbol{H}_3$)$_3$ rotamer B), 1.42 (5.4H, s, C(C$\boldsymbol{H}_3$)$_3$ rotamer A); δ_C (176 MHz, $CDCl_3$) 200.6 (***C***HO rotamer B), 200.4 (***C***HO rotamer A), 153.9 (***C***=O), 80.6 (***C***$(CH_3)_3$ rotamer A), 80.2 (***C***$(CH_3)_3$ rotamer B), 65.0 (***C***-2 rotamer A), 64.8 (***C***-2 rotamer B), 46.8 (***C***-5 rotamer B), 46.7 (***C***-5 rotamer A), 28.5 (***C***-3 rotamer A), 28.3 (***C***-3 rotamer B), 28.4 (C(***C***H_3)$_3$ rotamer B), 28.2 (C(***C***H_3)$_3$ rotamer A), 23.9 (***C***-4); *m/z* (LCMS, ESI^+) 200 (MH^+).

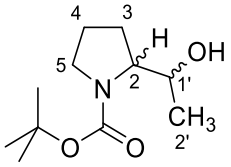
(*N*-*tert*-Butoxycarbonyl)-2-vinylpyrrolidine[223] 65



Procedure:[229] Methyltriphenylphosphonium bromide (98%, 4.77 g, 13.07 mmol) was dissolved in THF (26 mL) and cooled to −10 °C. Butyllithium (1.6 M in hexanes, 8.17 mL, 13.07 mmol) was added slowly. The resultant dark orange solution was stirred at RT for 30 min then re-cooled before the addition of aldehyde **(*S*)-64** (1.56 g, 7.83 mmol) in THF (8 mL) gave a dark red solution that was reacted at RT for 4 h. The reaction was quenched by addition of saturated $NH_4Cl_{(aq.)}$ (15 mL) before removal of volatiles under reduced pressure. The crude residue was diluted with H_2O (20 mL) and extracted with ether (3 × 20 mL). The combined organic extracts were washed with brine (20 mL) and saturated $NaHCO_{3(aq.)}$ (20 mL) before being dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography (5% → 20% ether in petrol) yielded the title compound (685 mg, 44%) as a clear colourless oil.

R_f 0.20 (20% EtOAc in petrol); ν_{max} (ATR) 2972 (w), 2933 (w), 2877 (w), 1689 (s), 1477 (w), 1453 (w), 1389 (s), 1365 (m), 1253 (w), 1166 (s), 1113 (m) cm^{-1}; δ_H (600 MHz, $CDCl_3$, mixture of rotamers) 5.81 – 5.64 (1H, m, 1′-***H***), 5.12 – 4.96 (2H, m, 2′-$\boldsymbol{H}_2$), 4.38 – 4.27 (0.4H, m, 2-***H*** rotamer B), 4.27 – 4.16 (0.6H, m, 2-***H*** rotamer A), 3.43 – 3.28 (2H, m, 5-$\boldsymbol{H}_2$), 2.07 – 1.92 (1H, m, 3-***H***H′), 1.87 – 1.74 (2H, m, 4-$\boldsymbol{H}_2$), 1.72 – 1.65 (1H, m, 3-H***H***′), 1.48 – 1.37 (9H, s, C(C$\boldsymbol{H}_3$)$_3$); δ_C (151 MHz, $CDCl_3$, mixture of rotamers) 154.7 (***C***=O), 138.9 (***C***-1′ rotamer A), 138.6 (***C***-1′ rotamer B), 113.6 (***C***-2′), 79.1 (***C***$(CH_3)_3$), 59.2 (***C***-2 rotamer A), 58.9 (***C***-2 rotamer B), 46.5 (***C***-5 rotamer B), 46.1 (***C***-5 rotamer A), 32.0 (***C***-3 rotamer A), 31.4 (***C***-3 rotamer B), 28.5 (C(***C***$H_3)_3$), 23.4 (***C***-4 rotamer B)), 22.7 (***C***-4 rotamer A)); *m/z* (LCMS, ESI^+) 142 (M − $C(CH_3)_3$ + $2H^+$), 183 (M – CH_3 + H^+).

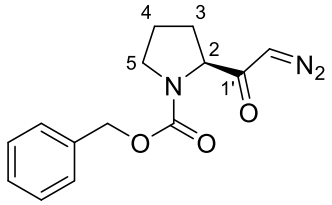
(*N*-*tert*-Butoxycarbonyl)-2-(1′-hydroxyethyl)pyrrolidine[279] **66**



Procedure B for the synthesis of alcohol **63** also formed the title compound (41 mg, 21% by ^1H NMR analysis) as a by-product.

R_f 0.23 (30% EtOAc in petrol); ν_{max} (ATR) 3439 (br), 2972 (m), 2929 (w), 2880 (w), 1667 (s), 1454 (w), 1400 (s), 1366 (m), 1253 (w), 1167 (s), 1111 (m) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 3.99 – 3.93 (1H, m, 2-***H***), 3.93 – 3.86 (1H, m, 1′-***H***), 3.55 – 3.48 (1H, m, 5-***H***H′), 3.23 (1H, dt, *J* = 10.5, 7.5 Hz, 5-H***H***′), 2.05 – 1.97 (1H, m, 3-***H***H′), 1.87 – 1.79 (1H, m, 4-***H***H′), 1.72 (1H, dquin, *J* = 12.5, 7.5 Hz, 4-H***H***′), 1.66 – 1.58 (1H, m, 3-H***H***′), 1.45 (9H, s, C(C$\boldsymbol{H}_3$)$_3$), 1.09 – 1.03 (3H, m, 2′-$\boldsymbol{H}_3$); δ_C (176 MHz, $CDCl_3$) 156.7 (***C***=O), 80.0 (***C***$(CH_3)_3$), 69.9 (***C***-1′), 63.5 (***C***-2), 48.2 (***C***-5), 28.4 (C(***C***$H_3)_3$), 28.1 (***C***-3), 24.1 (***C***-4), 17.4 (***C***-2′); *m/z* (LCMS, ESI^+) 238 (MNa^+).

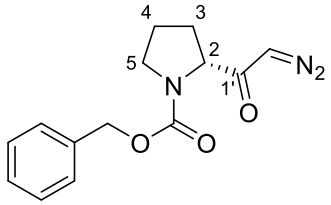
(2*S*)-2-(1′-Oxo-2′-diazoethyl)pyrrolidine-1-carboxylic acid benzyl ester[222] (*S*)-69



Procedure:[222] To a solution of (*S*)-*N*-Z-proline **(*S*)-68** (95%, 1.22 g, 4.65 mmol) in DCM (15 mL) at 0 °C was added oxalyl chloride (98%, 0.54 mL, 6.28 mmol) dropwise, followed by DMF (10 drops). The mixture was stirred at RT for 2 h prior to concentration under reduced pressure and the addition of THF (20 mL) and MeCN (20 mL). After cooling the solution in an ice bath, NEt_3 (1.30 mL, 9.30 mmol) and $TMSCHN_2$ (2 M solution in hexanes, 5.11 mL, 10.23 mmol) were added and the reaction mixture stirred for 5 h at 0 °C. The completed reaction was then quenched with AcOH (2 mL), diluted with H_2O (40 mL) and concentrated *in vacuo* before addition of EtOAc (30 mL) and washing with saturated $NaHCO_{3(aq.)}$ (3 × 20 mL). The organic phase was washed with brine (20 mL) and dried over $MgSO_4$ before evaporation of solvent under reduced pressure and column chromatography (0% → 40% EtOAc in hexanes) to furnish the title compound (967 mg, 76%) as a yellow oil.

R_f 0.20 (50% EtOAc in hexanes); $[\alpha]^{28}_D$ (c = 1.00 g/100 mL, $CHCl_3$) −135.8° (lit.:[231] $[\alpha]^{25}_D$ (c = 1.10 g/100 mL, $CHCl_3$) −131.8°); ν_{max} (ATR) 3068 (w), 3031 (w), 2953 (w), 2879 (w), 2101 (s), 1694 (s), 1639 (s), 1409 (s), 1351 (s), 1113 (m) cm^{-1}; δ_H (600 MHz, $CDCl_3$, mixture of rotamers) 7.40 – 7.27 (5H, m, Ar***H***), 5.48 (0.5H, s, C***H***N_2), 5.25 (0.5H, s, C***H***N_2), 5.21 – 5.04 (2H, m, PhC***H***$_2$), 4.37 –4.31 (0.5H, m, 2-***H***), 4.31 – 4.25 (0.5H, m, 2-***H***), 3.59 – 3.52 (1.5H, m, 5-***H***$_2$, 5-***H***H′), 3.53 – 3.45 (0.5H, m, 5-H***H***′), 2.22 – 2.15 (0.5H, m, 3-***H***H′), 2.13 – 2.01 (1.5H, m, 3-***H***$_2$, 3-H***H***′), 1.99 – 1.84 (2H, m, 4-***H***$_2$); δ_C (151 MHz, $CDCl_3$, mixture of rotamers) 195.4 (***C***-1′), 194.5 (***C***-1′), 155.4 (N***C***O_2), 154.7 (N***C***O_2), 136.6 (Ar***C***), 136.5 (Ar***C***), 128.6(3) (Ar***C***), 128.5(8) (Ar***C***), 128.2(4) (Ar***C***), 128.2(3) (Ar***C***), 128.1(7) (Ar***C***), 128.1 (Ar***C***), 67.4 (Ph***C***H_2), 64.2 (***C***-2), 64.1 (***C***-2), 53.5 (***C***HN_2), 52.8 (***C***HN_2), 47.5 (***C***-5), 47.1 (***C***-5), 31.4 (***C***-3), 29.8 (***C***-3), 24.5 (***C***-4), 23.7 (***C***-4); *m/z* (LCMS, ESI^+) 296 (MNa^+).

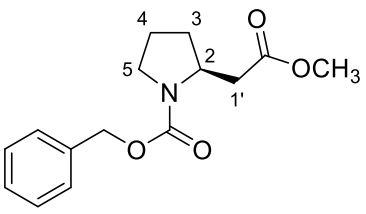
(2*R*)-2-(1′-Oxo-2′-diazoethyl)pyrrolidine-1-carboxylic acid benzyl ester[280] (*R*)-69



Procedure:[222] (*R*)-*N*-Z-proline **(*R*)-68** (2.08 g, 8.18 mmol) was reacted using the same conditions for the synthesis of diazoketone **(*S*)-69**, furnishing the title compound (1.60 g, 72%) as a yellow oil.

R_f 0.20 (50% EtOAc in hexanes); $[\alpha]^{28}_D$ (c = 1.00 g/100 mL, $CHCl_3$) +118.6° (lit.:[231] $[\alpha]^{25}_D$ (c = 1.10 g/100 mL, $CHCl_3$) −131.8° for (*S*) enantiomer); ν_{max} (ATR) 3066 (w), 3022 (w), 2951 (w), 2880 (w), 2101 (s), 1695 (s), 1641 (s), 1408 (s), 1351 (s), 1113 (m) cm^{-1}; δ_H (400 MHz, $CDCl_3$, mixture of rotamers) 7.40 – 7.27 (5H, m, Ar***H***), 5.48 (0.5H, s, C***H***N_2), 5.25 (0.5H, s, C***H***N_2), 5.21 – 5.04 (2H, m, PhC***H***$_2$), 4.37 – 4.31 (0.5H, m, 2-***H***), 4.31 – 4.25 (0.5H, m, 2-***H***), 3.59 – 3.52 (1.5H, m, 5-***H***$_2$, 5-***H***H′), 3.53 – 3.45 (0.5H, m, 5-H***H***′), 2.22 – 2.15 (0.5H, m, 3-***H***H′), 2.13 – 2.01 (1.5H, m, 3-***H***$_2$, 3-H***H***′), 1.99 – 1.84 (2H, m, 4-***H***$_2$); δ_C (101 MHz, $CDCl_3$, mixture of rotamers) 195.4 (***C***-1′), 194.5 (***C***-1′), 155.4 (N***C***O_2), 154.7 (N***C***O_2), 136.6 (Ar***C***), 136.5 (Ar***C***), 128.6(0) (Ar***C***), 128.5(7) (Ar***C***), 128.2 (Ar***C***), 128.1 (Ar***C***), 128.0 (Ar***C***), 67.4 (Ph***C***H_2), 64.2 (***C***-2), 64.1 (***C***-2), 53.5 (***C***HN_2), 52.8 (***C***HN_2), 47.5 (***C***-5), 47.1 (***C***-5), 31.4 (***C***-3), 29.8 (***C***-3), 24.5 (***C***-4), 23.7 (***C***-4); *m/z* (LCMS, ESI^+) 296 (MNa^+); Accurate mass: Found MNa^+, 296.1012: $C_{14}H_{15}N_3O_3Na$ requires *M*, 296.1006.

(2*S*)-2-(Methoxycarbonylmethyl)pyrrolidine-1-carboxylic acid benzyl ester[222] (*S*)-70

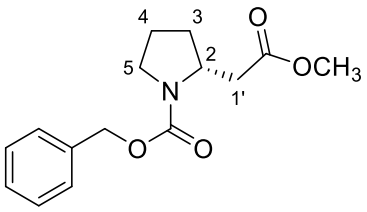


Procedure:[222] Diazoketone **(*S*)-69** (544 mg, 1.99 mmol) was dissolved in dry MeOH (6.4 mL) then treated dropwise with a solution of silver benzoate (46 mg, 0.20 mmol) in NEt_3 (0.83 mL, 5.97 mmol). The exothermic reaction mixture was stirred at RT for 3 h before being cooled in

an ice bath and quenched with aq. sodium metabisulfite (20%, 6.2 mL). The resulting silver salts were removed by filtration and washed with EtOAc (3 × 15 mL), then the organic filtrate washed with brine (30 mL) and dried over $MgSO_4$. Removal of solvent under reduced pressure and purification by SiO_2 column (0% → 50% EtOAc in hexanes) afforded the title compound (452 mg, 82%) as a clear colourless oil.

R_f 0.46 (50% EtOAc in hexanes); $[\alpha]^{28}_D$ (c = 1.00 g/100 mL, $CHCl_3$) −30.2° (lit.:[231] $[\alpha]^{25}_D$ (c = 1.33 g/100 mL, $CHCl_3$) −36.6°); ν_{max} (ATR) 2950 (w), 2879 (w), 1733 (m), 1693 (s), 1407 (m), 1356 (m), 1154 (m), 1095 (m) cm^{-1}; δ_H (600 MHz, $CDCl_3$, mixture of rotamers) 7.37 – 7.32 (4H, m, Ar***H***), 7.31 – 7.27 (1H, m, Ar***H***), 5.17 – 5.08 (2H, m, PhC***H***$_2$), 4.23 (1H, ddt, *J* = 9.5, 7.5, 3.5 Hz, 2-***H***), 3.64 (3H, s, CO_2C***H***$_3$), 3.48 – 3.38 (2H, m, 5-***H***$_2$), 3.01 – 2.91 (0.6H, m, 1′-***H***H′ rotamer A), 2.88 – 2.76 (0.4H, m, 1′-***H***H′ rotamer B), 2.34 (1H, apparent dd, *J* = 15.0, 9.5 Hz, 1′-H***H***′ rotamer A, 1′-H***H***′ rotamer B), 2.07 (1H, dq, *J* = 12.5, 8.0 Hz, 3-***H***H′), 1.91 – 1.81 (2H, m, 4-***H***$_2$), 1.79 – 1.73 (1H, m, 3-H***H***′); δ_C (151 MHz, $CDCl_3$, mixture of rotamers) 171.9 (***C***O_2CH_3), 154.7(N***C***O_2), 136.9 (Ar***C***), 128.5 (Ar***C***), 128.0 (Ar***C***), 127.9 (Ar***C***), 66.7 (Ph***C***H_2), 54.6 (***C***-2), 54.1 (***C***-2), 51.6 (CO_2***C***H_3), 46.8 (***C***-5), 46.5 (***C***-5), 39.2 (***C***-1′), 38.2 (***C***-1′), 31.4 (***C***-3), 30.7 (***C***-3), 23.6 (***C***-4), 22.8 (***C***-4); *m/z* (LCMS, ESI^+) 278 (MH^+), 300 (MNa^+).

(2*R*)-2-(Methoxycarbonylmethyl)pyrrolidine-1-carboxylic acid benzyl ester[280] (*R*)-70

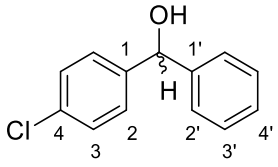


Procedure:[222] The conditions for the rearrangement of diazoketone **(*S*)-69** were applied to diazoketone **(*R*)-69** (630 mg, 2.31 mmol) furnishing the title compound (587 mg, 92%) as a clear colourless oil.

R_f 0.46 (50% EtOAc in hexanes); $[\alpha]^{27}_D$ (c = 1.00 g/100 mL, $CHCl_3$) +27.2° (lit.:[231] $[\alpha]^{25}_D$ (c = 1.33 g/100 mL, $CHCl_3$) −36.6° for (S)-isomer); ν_{max} (ATR) 2949 (w), 2875 (w), 1733 (m), 1693

(s), 1407 (s), 1356 (m), 1156 (m), 1095 (m) cm^{-1}; δ_H (400 MHz, $CDCl_3$, mixture of rotamers) 7.38 – 7.26 (5H, m, Ar***H***), 5.17 – 5.08 (2H, m, PhC***H***$_2$), 4.27 – 4.19 (1H, m, 2-***H***), 3.64 (3H, s, CO_2C***H***$_3$), 3.48 – 3.38 (2H, m, 5-***H***$_2$), 2.97 (0.6H, apparent br d, *J* = 14.5 Hz, 1'-***H***H' rotamer A), 2.79 (0.4H, apparent br d, *J* = 14.5 Hz, 1'-***H***H' rotamer B), 2.34 (1H, dd, *J* = 15.0, 9.5 Hz, 1'-H***H***' rotamer A, 1'-H***H***' rotamer B), 2.07 (1H, dq, *J* = 12.5, 8.0 Hz, 3-***H***H'), 1.91 – 1.81 (2H, m, 4-***H***$_2$), 1.79 – 1.73 (1H, m, 3-H***H***'); δ_C (101 MHz, $CDCl_3$, mixture of rotamers) 171.9 (***C***O_2CH_3), 154.7 (N***C***O_2), 136.9 (Ar***C***), 128.5 (Ar***C***), 128.0 (Ar***C***), 127.9 (Ar***C***), 66.7 (Ph***C***H_2), 54.6 (***C***-2), 54.1 (***C***-2), 51.6 (CO_2***C***H_3), 46.8 (***C***-5), 46.5 (***C***-5), 39.2 (***C***-1'), 38.2 (***C***-1'), 31.4 (***C***-3), 30.7 (***C***-3), 23.6 (***C***-4), 22.8 (***C***-4); *m/z* (LCMS, ESI^+) 278 (MH^+), 300 (MNa^+); Accurate mass: Found MH^+, 278.1393: $C_{15}H_{20}NO_4$ requires *M*, 278.1387.

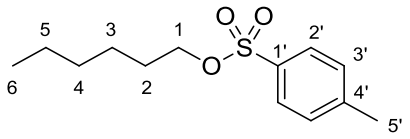
4-Chlorophenyl(phenyl)methanol[281] **73**



Procedure: 4-Chlorobenzaldehyde **72** (98%, 2.00 g, 13.94 mmol) was reacted according to general procedure C, furnishing the title compound (2.72 g, 89%) as a white solid after chromatography on silica (10% → 60% ether in petrol).

R_f 0.37 (25% ether in petrol); M.p. 59 – 60 °C (lit.:[282] 59 – 61 °C); ν_{max} (ATR) 3308 (br), 3062 (w), 3027 (w), 2885 (w), 1594 (w), 1486 (s), 1447 (m), 1408 (m), 1175 (m), 1085 (s), 1011 (s) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 7.35 – 7.33 (4H, m, Ar***H***), 7.31 – 7.30 (3H, m, Ar***H***), 7.30 – 7.27 (2H, m, Ar***H***), 5.80 (1H, s, Ar_2C***H***(OH)), 2.25 (1H, s, O***H***); δ_C (176 MHz, $CDCl_3$) 143.4 (***C***-1'), 142.2 (***C***-1), 133.3 (***C***-4), 128.6 (Ar***C***), 128.5 (Ar***C***), 127.9 (Ar***C***), 127.8 (Ar***C***), 126.5 (Ar***C***), 75.6 (Ar_2***C***H(OH)); *m/z* (LCMS, ESI^+) 201 ($M(^{35}Cl) - OH^-$), 203 ($M(^{37}Cl) - OH^-$).

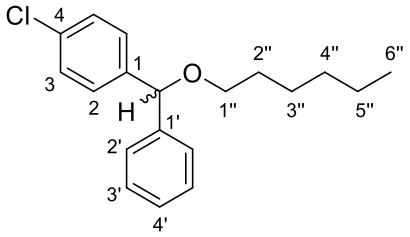
***p*-Toluenesulfonic acid hexyl ester[283] 74**



Procedure: The tosylation procedure employed in the synthesis of nitrile **(*S*)-61** was applied to hexan-1-ol (0.50 mL, 3.98 mmol). The crude material was not purified but instead treated with $NH_4OH_{(aq.)}$ (28% NH_3, 5 mL) with stirring for 30 min. The mixture was then separated and the organic layer washed with 1 M $HCl_{(aq.)}$ (2 × 5 mL) and brine (10 mL), dried over $MgSO_4$ and concentrated under reduced pressure to afford the title compound (1.02 g, quantitative) as a pale yellow oil.

R_f 0.36 (25% ether in petrol); ν_{max} (ATR) 2955 (w), 2930 (w), 2860 (w), 1598 (w), 1467 (w), 1359 (m), 1175 (s), 1097 (w) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 7.78 (2H, d, *J* = 8.0 Hz, 2′-***H***$_2$), 7.33 (2H, d, *J* = 8.0 Hz, 3′-***H***$_2$), 4.01 (2H, t, *J* = 7.0 Hz, 1-***H***), 2.44 (3H, s, 5′-***H***$_3$), 1.62 (2H, tt, *J* = 7.5, 7.0 Hz, 2-***H***$_2$), 1.28 (2H, quin, *J* = 7.5 Hz, 3-***H***$_2$), 1.23 (2H, tq, *J* = 7.5, 7.0 Hz, 5-***H***$_2$), 1.19 (2H, quin, *J* = 7.5 Hz, 4-***H***$_2$), 0.84 (3H, t, *J* = 7.0 Hz, 6-***H***$_3$); δ_C (176 MHz, $CDCl_3$) 144.6 (***C***-1′), 133.3 (***C***-4′), 129.7 (***C***-3′), 127.9 (***C***-2′), 70.7 (***C***-1), 31.1 (***C***-4), 28.8 (***C***-2), 25.0 (***C***-3), 22.4 (***C***-5), 21.6 (***C***-5′), 13.9 (***C***-6); *m/z* (GCMS, EI) 91 (M − $C_6H_{13}SO_3^{\bullet}$), 173 (M − $C_6H_{13}^{\bullet}$).

4-Chlorophenyl(phenyl)(hexyloxy)methane 75



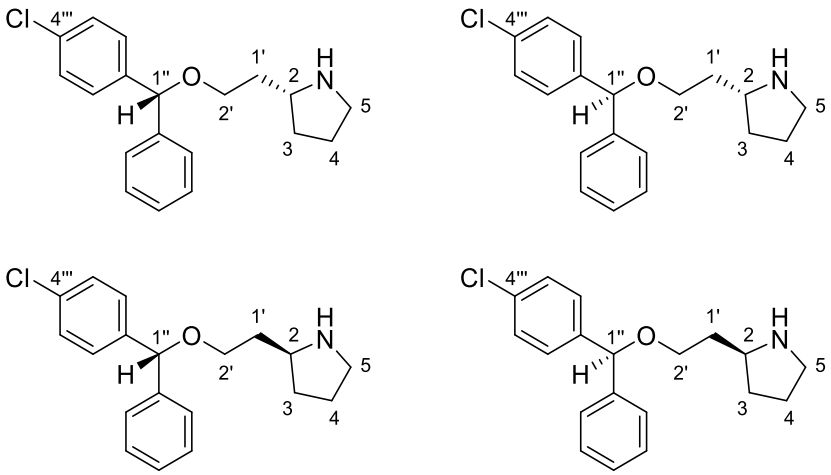
Procedure: A round bottomed flask fitted with a reflux condenser was charged with NaH (60%, 94 mg, 2.34 mmol) and toluene (4 mL). To this was added alcohol **73** (256 mg, 1.17 mmol) and the solution heated to reflux for 3 h. The reacton mixture was allowed to cool to RT and tosylate **74** (200 mg, 0.78 mmol) in toluene (1.3 mL) was added. The resultant mixture was heated to reflux for 20 h after which the reaction was cooled in an ice bath and quenched with H_2O (2 mL). The biphasic mixture was extracted with EtOAc (3 × 5 mL) and the combined

organic extracts washed with brine (10 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The resulting residue was then purified by chromatography on SiO_2 (0% → 20% ether in petrol) to furnish the *title compound* (193 mg, 55%) as a clear oil.

R_f 0.73 (25% ether in petrol); ν_{max} (ATR) 2953 (m), 2928 (m), 2856 (m), 1597 (w), 1489 (m), 1453 (w), 1089 (s), 1014 (m) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 7.32 – 7.24 (9H, m, Ar***H***), 5.28 (1H, s, Ar_2C***H***O), 3.42 (2H, apparent tt, *J* = 6.5, 3.0 Hz, 1''-$\boldsymbol{H}_2$), 1.62 (2H, tt, *J* = 7.5, 6.5 Hz, 2''-$\boldsymbol{H}_2$), 1.36 (2H, quin, *J* = 7.5 Hz, 3''-$\boldsymbol{H}_2$), 1.32 – 1.23 (4H, m, 4''-$\boldsymbol{H}_2$, 5''-$\boldsymbol{H}_2$), 0.87 (3H, t, *J* = 6.5 Hz, 6'-$\boldsymbol{H}_3$); δ_C (176 MHz, $CDCl_3$) 142.1 (***C***-1'), 141.3 (***C***-1), 133.0 (***C***-4), 128.5 (Ar***C***), 128.4 (Ar***C***), 128.2 (Ar***C***), 127.5 (Ar***C***), 126.9 (Ar***C***), 82.9 (Ar_2***C***HO), 69.3 (***C***-1''), 31.6 (***C***-4''), 29.8 (***C***-2''), 25.9 (***C***-3''), 22.6 (***C***-5''), 14.0 (***C***-6''); *m/z* (GCMS, EI) 201 ($M(^{35}Cl) - C_6H_{13}O^{\bullet}$), 203 ($M(^{37}Cl) - C_6H_{13}O^{\bullet}$).

2-(2'-[{4'''Chlorophenyl}{phenyl}methoxy]ethyl)pyrrolidine 77

A mixture of (2*R*,1''*R*), (2*R*,1''*S*), (2*S*,1''*R*) and (2*S*,1''*S*) diastereomers



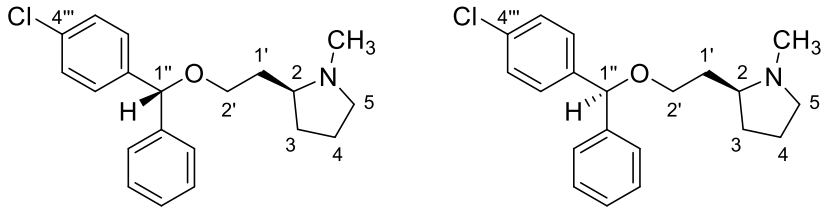
Procedure: General procedure B was used in the reaction of alcohol **73** (99 mg, 0.45 mmol) and racemic homoprolinol **63** (100 mg, 0.45 mmol). Silica flash column chromatography (0% → 5% MeOH in $CHCl_3$ with 1% NEt_3), furnished the *title compound* (94 mg, 66%) as a clear light brown oil.

R_f 0.35 (10% MeOH and 1% $NH_4OH_{(aq.)}$ in $CHCl_3$); ν_{max} (ATR) 3368 (br), 2938 (m), 2866 (m), 1489 (m), 1452 (w), 1400 (w), 1181 (w), 1087 (s), 1012 (m) cm^{-1}; δ_H (700 MHz, $CDCl_3$ mixture of

diastereomers) 7.33 – 7.27 (4H, m, Ar***H***), 7.27 – 7.23 (5H, m, Ar***H***), 5.30 (1H, s, 1''-***H***), 4.30 – 4.10 (1H, m, N***H***), 3.55 – 3.49 (2H, m, 2'-$\boldsymbol{H}_2$), 3.31 – 3.25 (1H, m, 2-***H***), 3.06 (1H, dddd, *J* = 10.5, 9.0, 8.0, 6.0 Hz, 5-***H***H'), 2.93 (1H, dtd, *J* = 10.5, 8.5, 6.5 Hz, 5-H***H***'), 1.96 – 1.87 (2H, m, 3-***H***H', 1'-***H***H'), 1.86 – 1.78 (2H, m, 4-***H***H', 1'-H***H***'), 1.78 – 1.71 (1H, m, 4-H***H***'), 1.41 – 1.34 (1H, m, 3-H***H***'); δ_C (176 MHz, $CDCl_3$, mixture of diastereomers) 142.0 (Ar***C***), 141.9 (Ar***C***), 141.1(1) (Ar***C***), 141.0(5) (Ar***C***), 133.3 (Ar***C***), 133.2 (Ar***C***), 128.7 (Ar***C***), 128.6(3) (Ar***C***), 128.6(1) (Ar***C***), 128.3(8) (Ar***C***), 128.3(7) (Ar***C***), 127.7(9) (Ar***C***), 127.7(7) (Ar***C***), 127.0(3) (Ar***C***), 127.0(0) (Ar***C***), 83.2(2) (***C***-1''), 83.2(0) (***C***-1''), 67.3 (***C***-2'), 67.2 (***C***-2'), 57.1(7) (***C***-2), 57.1(5) (***C***-2), 46.1(3) (***C***-5), 46.1(2) (***C***-5), 35.4 (***C***-1'), 35.3 (***C***-1'), 31.6(0) (***C***-3), 31.5(7) (***C***-3), 25.0 (***C***-4), 24.9 (***C***-4); *m/z* (LCMS, ESI^+) 316 ($M(^{35}Cl)H^+$), 318 ($M(^{37}Cl)H^+$); Accurate mass: Found MH^+, 316.1467: $C_{19}H_{23}NO^{35}Cl$ requires *M*, 316.1468.

2-(2'-[{4'''-Chlorophenyl}{phenyl}methoxy]ethyl)-1-methylpyrrollidine 78A

A mixture of (2*S*,1''*R*) and (2*S*,1''*S*) diastereomers



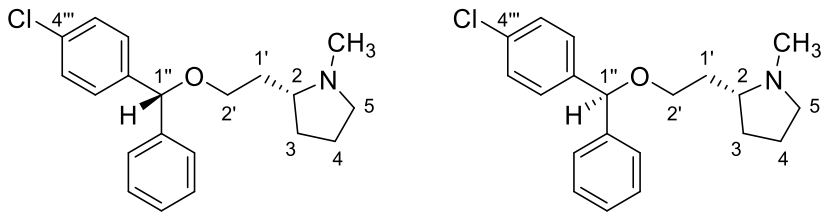
Procedure: General procedure B was used in the reaction of alcohol **73** (109 mg, 0.50 mmol) and homoprolinol **(*S*)-57** (65 mg, 0.50 mmol). Silica flash column chromatography (0% → 10% MeOH in $CHCl_3$ with 1% NEt_3), furnished the *title compound* (56 mg, 34%) as a thick, clear colourless oil.

R_f 0.36 (10% MeOH and 1% NEt_3 in $CHCl_3$); ν_{max} (ATR) 2946 (m), 2940 (w), 2771 (m), 1488 (m), 1452 (m), 1086 (s), 1013 (m) cm^{-1}; δ_H (400 MHz, $CDCl_3$, mixture of diastereomers) 7.28 – 7.16 (9H, m, Ar***H***), 5.22 (1H, s, 1''-***H***), 3.48 – 3.35 (2H, m, 2'-$\boldsymbol{H}_2$), 3.07 – 3.00 (1H, m, 5-***H***H'), 2.27 (3H, s, NC$\boldsymbol{H}_3$), 2.22 – 1.97 (3H, m, 2-***H***, 5-H***H***', 1'-***H***H'), 1.91 – 1.79 (1H, m, 3-***H***H'), 1.77 – 1.67 (1H, m, 4-***H***H'), 1.66 – 1.58 (1H, m, 4-H***H***'), 1.57 – 1.47 (1H, m, 1'-H***H***'), 1.46 – 1.36 (1H, m,

3-H***H***'); δ_C (101 MHz, $CDCl_3$, mixture of diastereomers) 142.0(9) (Ar***C***), 142.0(5) (Ar***C***), 141.2(2) (Ar***C***), 141.1(9) (Ar***C***), 133.2(4) (Ar***C***), 133.2(1) (Ar***C***), 128.6(4) (Ar***C***), 128.6(3) (Ar***C***), 128.6(0) (Ar***C***), 128.4 (Ar***C***), 128.3 (Ar***C***), 127.8 (Ar***C***), 127.7 (Ar***C***), 127.0(3) (Ar***C***), 126.9(7) (Ar***C***), 83.2(4) (***C***-1''), 83.2(2) (***C***-1''), 67.2 (***C***-2'), 67.1 (***C***-2'), 64.2 (***C***-2), 57.2 (***C***-5), 40.5 (N***C***H_3), 33.9 (***C***-1'), 31.0 (***C***-3), 30.9 (***C***-3), 22.0 (***C***-4); *m/z* (LCMS, ESI^+) 330 ($M(^{35}Cl)H^+$), 332 ($M(^{37}Cl)H^+$); Accurate mass: Found MH^+, 330.1617: $C_{20}H_{25}NO^{35}Cl$ requires *M*, 330.1625.

2-(2'-[{4'''-Chlorophenyl}{phenyl}methoxy]ethyl)-1-methylpyrrollidine 78B

A mixture of (2*R*,1''*R*) and (2*R*,1''*S*) diastereomers



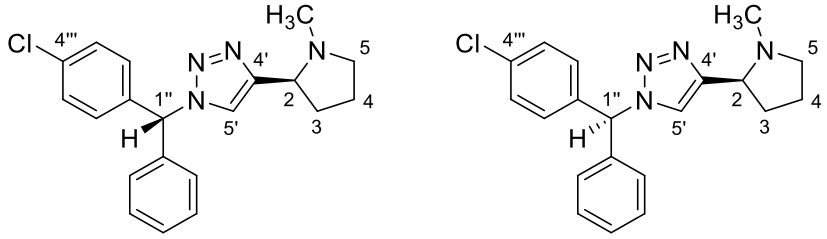
Procedure: General procedure B was used in the reaction of alcohol **73** (109 mg, 0.50 mmol) and homoprolinol **(*R*)-57** (65 mg, 0.50 mmol). Silica flash column chromatography (0% → 10% MeOH in $CHCl_3$ with 1% NEt_3), furnished the *title compound* (94 mg, 57%) as a thick, clear colourless oil.

R_f 0.36 (10% MeOH and 1% NEt_3 in $CHCl_3$); ν_{max} (ATR) 3026 (w), 2938 (m), 2840 (w), 2774 (m), 1488 (m), 1452 (m), 1087 (s), 1013 (m) cm^{-1}; δ_H (700 MHz, $CDCl_3$, mixture of diastereomers) 7.33 – 7.22 (9H, m, Ar***H***), 5.28 (1H, s, Ar_2C***H***O), 3.52 – 3.42 (2H, m, 2'-***H***$_2$), 3.07 – 3.03 (1H, m, 5-***H***H'), 2.30 (3H, s, N***C***H_3), 2.18 – 2.04 (3H, m, 2-***H***, 5-H***H***', 1'-***H***H'), 1.92 – 1.86 (1H, m, 3-***H***H'), 1.77 – 1.70 (1H, m, 4-***H***H'), 1.68 – 1.62 (1H, m, 4-H***H***'), 1.57 – 1.50 (1H, m, 1'-H***H***'), 1.48 – 1.41 (1H, m, 3-H***H***'); δ_C (176 MHz, $CDCl_3$, mixture of diastereomers) 142.2 (Ar***C***), 142.1 (Ar***C***), 141.2(8) (Ar***C***), 141.2(5) (Ar***C***), 133.2(2) (Ar***C***), 133.1(9) (Ar***C***), 128.6(4) (Ar***C***), 128.6(3) (Ar***C***), 128.6(0) (Ar***C***), 128.4 (Ar***C***), 128.3 (Ar***C***), 127.8 (Ar***C***), 127.7 (Ar***C***), 127.0(3) (Ar***C***), 126.9(8) (Ar***C***), 83.2(3) (Ar_2***C***HO), 83.2(1) (Ar_2***C***HO), 67.2(9) (***C***-2'), 67.2(6) (***C***-2'), 63.9(4) (***C***-2), 63.9(3) (***C***-2), 57.3 (***C***-5),

40.6 (N***C***H_3), 34.2 (***C***-1′), 31.1 (***C***-3), 31.0 (***C***-3), 22.1 (***C***-4); *m/z* (LCMS, ESI^+) 330 ($M(^{35}Cl)H^+$), 332 ($M(^{37}Cl)H^+$); Accurate mass: Found MH^+, 330.1619: $C_{20}H_{25}NO^{35}Cl$ requires *M*, 330.1625.

(*N*-Methyl)-2-(1′-[{4‴-chlorophenyl}{phenyl}methyl]-1′,2′,3′-triazol-4′-yl)pyrrolidine 83A

A mixture of (2*S*,1″*R*) and (2*S*,1″*S*) diastereomers

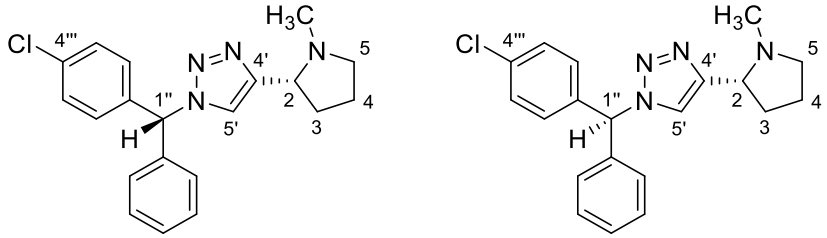


Procedure:[241] To a suspension of $LiAlH_4$ (95%, 45 mg, 1.12 mmol) in ether (4.5 mL), cooled in an ice bath, was added triazole **(*S*)-87** (123 mg, 0.28 mmol). The reaction mixture was allowed to warm to RT and stirred for 2 h, after which the solution was re-cooled to 0 °C quenched according to Fieser's method (general procedure D).[273] The residue was purified by column chromatography (0% → 5% EtOH in petrol with 5% EtOAc and 10% NEt_3) to furnish the *title compound* (34 mg, 34%) as a white solid.

R_f 0.22 (5% EtOH, 5% EtOAc and 10% NEt_3 in petrol); M.p. 100 – 103 °C; ν_{max} (ATR) 2971 (m), 2933 (m), 2876 (m), 2784 (m), 1491 (m), 1438 (m) cm^{-1}; δ_H (700 MHz, $CDCl_3$, mixture of diastereomers) 7.37 – 7.30 (6H, m, Ar***H***), 7.10 – 7.07 (3H, m, Ar***H***, 1″-***H*** isomer 1), 7.04 – 7.00 (2H, m, Ar***H***, 1″-***H*** isomer 2), 3.44 (1H, t, *J* = 8.0 Hz, 2-***H***), 3.15 (1H, apparent t, *J* = 8.5 Hz, 5-***H***H′), 2.32 – 2.24 (2H, m, 5-H***H***′, 3-***H***H′), 2.26 (3H, s, NC***H***$_3$), 1.94 – 1.86 (1H, m, 4-***H***H′), 1.86 – 1.77 (2H, m, 3-H***H***′, 4-H***H***′); δ_C (176 MHz, $CDCl_3$, mixture of diastereomers) 150.4 (***C***-4′ isomer 1), 150.1 (***C***-4′ isomer 2), 138.3 (Ar***C***), 137.7 (Ar***C***), 136.9 (Ar***C***), 134.5 (Ar***C***), 129.4 (Ar***C***), 129.3 (Ar***C***), 129.1 (Ar***C***), 129.0 (Ar***C***), 128.9 (Ar***C***), 128.7 (Ar***C***), 128.5 (Ar***C***), 128.4 (Ar***C***), 128.1 (Ar***C***), 128.0 (Ar***C***), 120.6 (***C***-5′ isomer 1), 120.5 (***C***-5′ isomer 2), 68.1 (***C***-1″ isomer 1), 67.4 (***C***-1″ isomer 2), 62.8 (***C***-2 isomer 1), 62.7 (***C***-2 isomer 2), 56.8 (***C***-5), 40.6 (***C***-6), 33.2 (***C***-3), 22.3 (***C***-4); *m/z* (LCMS, ESI^+) 353 ($M(^{35}Cl)H^+$), 355 ($M(^{37}Cl)H^+$); Accurate mass: Found MH^+, 353.1530: $C_{20}H_{22}N_4{}^{35}Cl$ requires *M*, 353.1533.

(*N*-Methyl)-2-(1′-[{4‴-chlorophenyl}{phenyl}methyl]-1′,2′,3′-triazol-4′-yl)pyrrolidine 83B

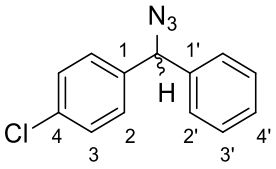
A mixture of (2*R*,1″*R*) and (2*R*,1″*S*) diastereomers



Procedure:[241] To a suspension of $LiAlH_4$ (95%, 82 mg, 2.05 mmol) in ether (5 mL), cooled in an ice bath, was added triazole **(*R*)-87** (143 mg, 0.33 mmol). The reaction mixture was allowed to warm to RT and stirred for 2 h, after which the solution was re-cooled to 0 °C and quenched according to Fieser's method (general procedure D).[273] The residue was purified by column chromatography (0% → 5% EtOH in petrol with 5% EtOAc and 10% NEt_3) to furnish the *title compound* (17 mg, 15%) as a yellow oil.

R_f 0.22 (5% EtOH, 5% EtOAc and 10% NEt_3 in petrol); ν_{max} (ATR) 2950 (m), 2840 (m), 2773 (m), 1491 (s), 1451 (m), 1210 (m), 1090 (s), 1041 (s) cm^{-1}; δ_H (400 MHz, $CDCl_3$, mixture of diastereomers) 7.38 – 7.29 (6H, m, Ar***H***), 7.12 – 7.00 (5H, m, Ar***H***, 1″-***H***), 3.46 (1H, t, *J* = 8.0 Hz, 2-***H***), 3.16 (1H, ddd, *J* = 10.0, 8.5, 2.5 Hz, 5-***H***H′), 2.36 – 2.22 (5H, m, 3-***H***H′, 5-H***H***′, NC$\boldsymbol{H}_3$), 1.96 – 1.77 (3H, m, 3-H***H***′, 4-$\boldsymbol{H}_2$); δ_C (101 MHz, $CDCl_3$, mixture of diastereomers) 150.4 (***C***-4′), 138.4 (Ar***C***), 137.9 (Ar***C***), 137.0 (Ar***C***), 134.6 (Ar***C***), 129.6 (Ar***C***), 129.5 (Ar***C***), 129.2(3) (Ar***C***), 129.1(8) (Ar***C***), 129.0 (Ar***C***), 128.9(0) (Ar***C***), 128.8(7) (Ar***C***), 128.6(2) (Ar***C***), 128.6(0) (Ar***C***), 128.3 (Ar***C***), 128.2 (Ar***C***), 120.8 (***C***-5′), 67.6 (***C***-1″), 62.9 (***C***-2), 57.0 (***C***-5), 40.7 (N***C***H_3), 33.3 (***C***-3), 22.5 (***C***-4); *m/z* (LCMS, ESI^+) 353 ($M(^{35}Cl)H^+$), 355 ($M(^{37}Cl)H^+$); Accurate mass: Found MH^+, 353.1527: $C_{20}H_{22}N_4{}^{35}Cl$ requires *M*, 353.1533.

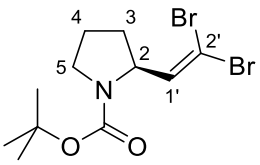
4-Chlorophenyl(phenyl)azidomethane[284] 84



Procedure:[235] To a 0 °C solution of $TMSN_3$ (94%, 2.82 mL, 20.04 mmol), $TsOH{\cdot}H_2O$ (98%, 1.30 g, 6.68 mmol) and $BF_3{\cdot}Et_2O$ (1.68 mL, 13.36 mmol) in toluene (17 mL) was slowly added a solution of alcohol **73** (1.46 g, 6.68 mmol) in toluene (17 mL). The reaction mixture was stirred at 0 °C for 30 min, after which TLC analysis revealed consumption of the starting material. The mixture was quenched with the addition of saturated $NaHCO_{3(aq.)}$ (15 mL) and extracted with EtOAc (3 × 20 mL). The combined organic extracts were washed with saturated $NaHCO_{3(aq.)}$ (30 mL) and brine (30 mL), then concentrated *in vacuo* before purification by chromatography on SiO_2 (10% → 20% ether in petrol) to afford the title compound (1.47 g, 90%) as a pale yellow oil.

R_f 0.61 (25% ether in petrol); ν_{max} (ATR) 3083 (w), 3058 (w), 3023 (w), 2095 (s), 1594 (w), 1486 (m), 1447 (m), 1244 (m), 1089 (m), 1011 (m) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 7.39 – 7.35 (2H, m, 3'-***H***$_2$), 7.34 – 7.31 (3H, m, 3-***H***$_2$, 4'-***H***), 7.28 (2H, d, *J* = 7.5 Hz, 2'-***H***$_2$), 7.25 (2H, d, *J* = 8.5 Hz, 2-***H***$_2$), 5.68 (1H, s, $Ar_2C\boldsymbol{H}(N_3)$); δ_C (176 MHz, $CDCl_3$) 139.1 (***C***-1'), 138.2 (***C***-1), 133.9 (***C***-4), 128.9 (***C***-3'), 128.8 (***C***-3), 128.7 (***C***-2), 128.3 (***C***-4'), 127.4 (***C***-2'), 67.8 ($Ar_2\boldsymbol{C}H(N_3)$); *m/z* (LCMS, ESI^+) 216 ($M(^{35}Cl) - N_2 + H^+$), 218 ($M(^{37}Cl) - N_2 + H^+$).

(2*S*)-(*N*-*tert*-Butoxycarbonyl)-2-(2',2'-dibromoethenyl)pyrrolidine[228] (*S*)-85

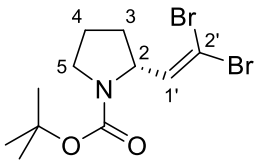


Procedure:[228] A solution of aldehyde **(*S*)-64** (1.44 g, 7.23 mmol) in DCM (14 mL) was added dropwise to a 0 °C solution of PPh_3 (7.58 g, 28.91 mmol) and CBr_4 (4.79 g, 14.45 mmol) in DCM (120 mL). The resulting solution was stirred at RT for 30 min before being poured into cooled saturated $NaHCO_{3(aq.)}$ (100 mL) and separated. The aqueous phase was washed with DCM (20 mL) and the combined organic extracts dried over $MgSO_4$ and concentrated under reduced

pressure. The crude residue was purified by column chromatography (40:1:1 → 10:1:1, petrol : EtOAc : EtOH) to afford the title compound (1.52 g, 59%) as an off-white solid.

R_f 0.45 (19:1:1, petrol : EtOAc : EtOH); M.p. 62 – 64 °C (lit.:[228] 58 – 59 °C); $[\alpha]^{21}_D$ (c = 1.00 g/100 mL, $CHCl_3$) +8.5° (lit.:[228] $[\alpha]^{26}_D$ (c = 0.89 g/100 mL, DCM) +24.0°); ν_{max} (ATR) 2976 (m), 2933 (w), 2872 (w), 1689 (s), 1607 (w), 1391 (s), 1365 (m), 1244 (m), 1162 (s), 1102 (m) cm^{-1}; δ_H (600 MHz, $CDCl_3$, mixture of rotamers) 6.38 – 6.25 (1H, m, 1′-***H***), 4.43 – 4.34 (0.3H, m, 2-***H*** rotamer B), 4.33 – 4.25 (0.7H, m, 2-***H*** rotamer A), 3.44 – 3.35 (0.7H, m, 5-***H***H′ rotamer A), 3.32 (1.3H, apparent dt, *J* = 10.5, 7.0 Hz, 5-H***H***′ rotamer A, 5-$\boldsymbol{H}_2$ rotamer B), 2.14 – 2.06 (1H, m, 3-***H***H′), 1.82 – 1.75 (2H, m, 4-$\boldsymbol{H}_2$), 1.70 – 1.63 (1H, m, 3-H***H***′), 1.40 (9H, s, C(C$\boldsymbol{H}_3$)$_3$); δ_C (151 MHz, $CDCl_3$) 154.3 (***C***=O), 140.4 (***C***-1′), 88.2 (***C***-2′), 79.8 (***C***$(CH_3)_3$), 59.5 (***C***-2), 46.4 (***C***-5), 31.8 (***C***-3), 28.5 (C(***C***$H_3)_3$), 23.6 (***C***-4); *m/z* (LCMS, ESI^+) 354 (M($^{79}Br_2$)H^+), 356 (M($^{79}Br^{81}Br$)H^+), 358 (M($^{81}Br_2$)H^+).

(2*R*)-(*N*-*tert*-Butoxycarbonyl)-2-(2′,2′-dibromoethenyl)pyrrolidine[228] (*R*)-85

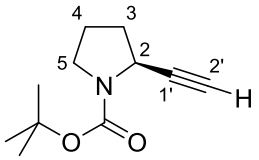


Procedure:[228] *N*-Boc-D-prolinol **(*R*)-59** (98%, 1.97 g, 9.59 mmol) was subjected to Parikh-Doering oxidation (see Procedure B for the synthesis of **(*S*)-64**), furnishing the crude aldehyde **(*R*)-64** (1.47 g, 77%) as a pale yellow oil which was used without further purification.

A solution of (*R*)-*N*-Boc-prolinal **(*R*)-64** (618 mg, 3.10 mmol) in DCM (3 mL) was added dropwise to a 0 °C solution of PPh_3 (3.25 g, 12.41 mmol) and CBr_4 (2.10 g, 6.20 mmol) in DCM (48 mL). The resulting solution was stirred at RT for 30 min before being poured into cooled saturated $NaHCO_{3(aq.)}$ (50 mL) and separated. The aqueous phase was washed with DCM (20 mL) and the combined organic extracts dried over $MgSO_4$ and concentrated under reduced pressure. The crude residue was purified by column chromatography (0% → 30% EtOAc in hexanes) to afford the title compound (738 mg, 67%) as an off-white solid.

R_f 0.45 (0.5% EtOAc and 0.5% EtOH in petrol); $[\alpha]^{28}_D$ (c = 1.00 g/100 mL, $CHCl_3$) −32.6° (lit.:[228] $[\alpha]^{26}_D$ (c = 1.15 g/100 mL, CH_2Cl_2) −24.0°); ν_{max} (ATR) 2973 (m), 2875 (w), 1693 (s), 1390 (s), 1241 (w), 1163 (m), 1107 (m) cm^{-1}; δ_H (700 MHz, $CDCl_3$, mixture of rotamers) 6.44 – 6.30 (1H, m, 1′-***H***), 4.50 – 4.28 (1H, m, 2-***H***), 3.51 – 3.42 (0.7H, m, 5-$\boldsymbol{H}_2$ rotamer B), 3.42 – 3.35 (1.3H, m, 5-$\boldsymbol{H}_2$ rotamer A), 2.17 (1H, dq, *J* = 14.0, 7.0 Hz, 3-***H***H′), 1.89 – 1.80 (2H, m, 4-$\boldsymbol{H}_2$), 1.76 – 1.70 (1H, m, 3-H***H***′), 1.47 (9H, s, C(C$\boldsymbol{H}_3$)$_3$); δ_C (176 MHz, $CDCl_3$) 154.5 (***C***=O), 140.6 (***C***-1′), 88.5 (***C***-2′), 79.9 (***C***$(CH_3)_3$), 59.7 (***C***-2), 46.6 (***C***-5), 32.0 (***C***-3), 28.7 (C(***C***$H_3)_3$), 23.8 (***C***-4); *m/z* (LCMS, ESI^+) 354 ($M(^{79}Br_2)H^+$), 356 ($M(^{79}Br^{81}Br)H^+$), 358 ($M(^{81}Br_2)H^+$).

(2*S*)-(*N*-*tert*-Butoxycarbonyl)-2-ethynylpyrrolidine[228] (*S*)-86

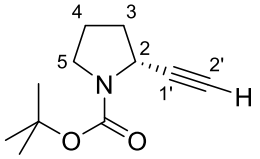


Procedure:[228] To a solution of dibromoethenyl compound **(*S*)-85** (1.33 g, 3.75 mmol) in THF (33 mL), cooled to −78 °C, was added butyllithium (1.6 M in hexanes, 24.70 mL, 7.49 mmol). The reaction mixture was stirred at −78 °C for 1 h, when consumption of starting material was observed by TLC, then quenched by the addition of saturated $NH_4Cl_{(aq.)}$ (30 mL). The mixture was allowed to warm to RT, after which THF was removed under reduced pressure before extraction with ether (3 × 20 mL). The combined organic extracts were washed with brine (30 mL), dried over $MgSO_4$ and concentrated *in vacuo* prior to purification by SiO_2 column (10% → 30% EtOAc in petrol) to furnish the title compound (549 mg, 75%) as a yellow oil.

R_f 0.31 (19:1:1, petrol : EtOAc : EtOH); $[\alpha]^{27}_D$ (c = 1.00 g/100 mL, $CHCl_3$) −49.5° (lit.:[236] $[\alpha]^{25}_D$ (c = 1.35 g/100 mL, $CHCl_3$) −66.3°); ν_{max} (ATR) 3304 (w), 3239 (w), 2971 (m), 2872 (w), 1689 (s), 1392 (s), 1365 (m), 1244 (m), 1158 (s), 1115 (m) cm^{-1}; δ_H (700 MHz, $CDCl_3$, mixture of rotamers) 4.50 – 4.42 (0.4H, m, 2-***H*** rotamer B), 4.40 – 4.33 (0.6H, m, 2-***H*** rotamer A), 3.46 – 3.38 (1H, m, 5-***H***H′), 3.31 – 3.23 (1H, m, 5-H***H***′), 2.21 – 2.15 (1H, m, 2′-***H***), 2.08 – 2.00 (2H, m, 3-***H***H′, 4-***H***H′), 2.00 – 1.96 (1H, m, 3-H***H***′), 1.89 – 1.83 (1H, m, 4-H***H***′), 1.44 (9H, s, C(C$\boldsymbol{H}_3$)$_3$); δ_C (176 MHz, $CDCl_3$, mixture of rotamers) 154.0 (***C***=O), 84.4 (***C***-1′ rotamer A), 84.1 (***C***-1′ rotamer

B), 79.7 (***C***$(CH_3)_3$), 69.8 (***C***-2′ rotamer B), 69.4 (***C***-2′ rotamer A), 48.0 (***C***-2 rotamer A), 47.7 (***C***-2 rotamer B), 45.9 (***C***-5 rotamer B), 45.4 (***C***-5 rotamer A), 33.6 (***C***-3 rotamer A), 32.9 (***C***-3 rotamer B), 28.4 (C(***C***$H_3)_3$), 24.4 (***C***-4 rotamer B), 23.5 (***C***-4 rotamer A); *m/z* (LCMS, ESI^+) 218 (MNa^+).

(2*R*)-(*N*-*tert*-Butoxycarbonyl)-2-ethynylpyrrolidine[285] (*R*)-86

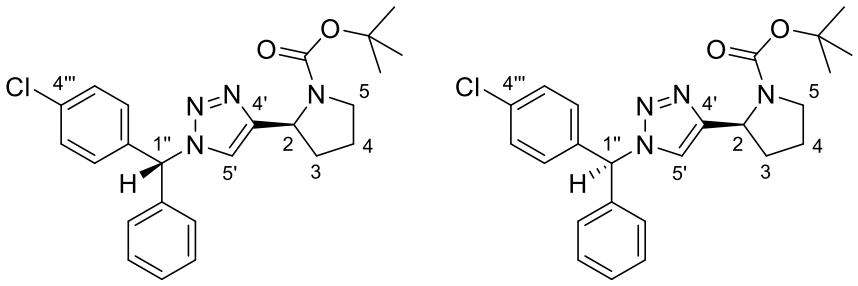


Procedure:[228] To a solution of dibromoethenyl compound **(*R*)-85** (710 mg, 2.00 mmol) in THF (18 mL), cooled to −78 °C, was added butyllithium (1.6 M in hexanes, 2.5 mL, 4.00 mmol). The reaction mixture was stirred at −78 °C for 1 h, when consumption of starting material was observed by TLC, then quenched by the addition of saturated $NH_4Cl_{(aq.)}$ (20 mL). The mixture was allowed to warm to RT, after which THF was removed under reduced pressure before extraction with ether (3 × 10 mL). The combined organic extracts were washed with brine (20 mL), dried over $MgSO_4$ and concentrated *in vacuo* prior to purification by SiO_2 column (10% → 20% EtOAc in petrol) to furnish the title compound (300 mg, 77%) as a yellow oil.

R_f 0.31 (0.5% EtOAc and 0.5% EtOH in petrol); $[\alpha]^{29}_D$ (c = 1.00 g/100 mL, $CHCl_3$) +65.3° (lit.:[285] $[\alpha]^{26}_D$ (c = 0.94 g/100 mL, MeOH) +113.0°); ν_{max} (ATR) 3293 (w), 3244 (w) 2975 (m), 2875 (w), 1690 (s), 1390 (s), 1366 (m), 1252 (m), 1162 (s), 1121 (m), 1091 (w) cm^{-1}; δ_H (700 MHz, $CDCl_3$, mixture of rotamers) 4.55 – 4.47 (0.4H, m, 2-***H*** rotamer B), 4.46 – 4.37 (0.6H, m, 2-***H*** rotamer A), 3.51 – 3.40 (1H, m, 5-***H***H′), 3.35 – 3.25 (1H, m, 5-H***H***′), 2.25 – 2.17 (1H, m, 2′-***H***), 2.12 – 1.98 (3H, m, 3-$\boldsymbol{H}_2$, 4-***H***H′), 1.94 – 1.86 (1H, m, 4-H***H***′), 1.47 (9H, s, C(C$\boldsymbol{H}_3)_3$); δ_C (176 MHz, $CDCl_3$, mixture of rotamers) 154.2 (***C***=O), 84.6 (***C***-1′ rotamer B), 84.3 (***C***-1′ rotamer A), 79.9 (***C***$(CH_3)_3$), 70.0 (***C***-2′ rotamer B), 69.6 (***C***-2′ rotamer A), 48.2 (***C***-2 rotamer B), 47.9 (***C***-2 rotamer A), 46.1 (***C***-5 rotamer B), 45.6 (***C***-5 rotamer A), 33.8 (***C***-3 rotamer B), 33.1 (***C***-3 rotamer A), 28.6 (C(***C***$H_3)_3$), 24.6 (***C***-4 rotamer B), 23.8 (***C***-4 rotamer A); *m/z* (LCMS, ESI^+) 196 (MH^+).

(*N*-*tert*-Butoxycarbonyl)-2-(1′-[{4‴-chlorophenyl}{phenyl}methyl]-1′,2′,3′-triazol-4′-yl)pyrrolidine 87A

A mixture of (2*S*,1″*R*) and (2*S*,1″*S*) diastereomers

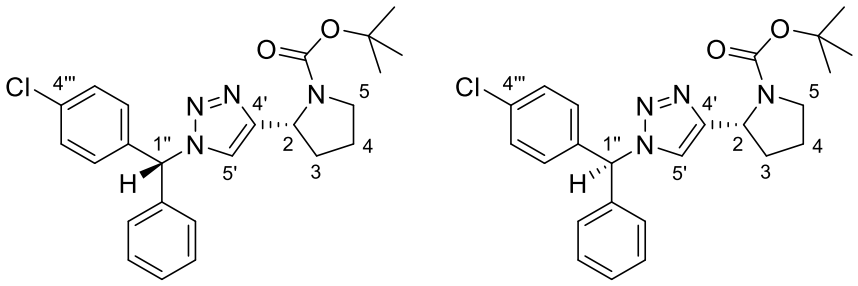


Procedure:[238] A microwave vial was charged with azide **84** (139 mg, 0.57 mmol), alkyne **(*S*)-86** (98 mg, 0.50 mmol), $CuSO_4{\cdot}5H_2O_{(aq.)}$ (1 M, 0.20 mL, 0.20 mmol), freshly prepared Na ascorbate$_{(aq.)}$ (1 M, 1.00 mL, 1.00 mmol), H_2O (1 mL) and MeOH (1 mL). The vial was sealed and reacted at 60 °C for 40 min, with a 30 s pre-mixing time. The mixture was then cooled, diluted with H_2O (5 mL) and extracted with DCM (3 × 10 mL) and the combined organic extracts washed with brine (10 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The crude residue was then purified by column chromatography (50% → 70% ether in petrol) to provide the *title compound* (153 mg, 70%) as a white solid.

R_f 0.10 (50% ether in petrol); M.p. 56 – 58 °C; ν_{max} (ATR) 2976 (w), 2928 (w), 2881 (w), 2017 (w), 1676 (s), 1490 (m), 1452 (w), 1391 (s), 1248 (w), 1162 (m), 1110 (w) cm^{-1}; δ_H (500 MHz, $(CD_3)_2SO$, 90 °C, mixture of diastereomers) 7.71 (1H, apparent d, *J* = 1.0 Hz, 1′′-***H***), 7.42 (2H, d, *J* = 8.5 Hz, Ar***H***), 7.40 – 7.34 (3H, m, Ar***H***), 7.24 – 7.18 (5H, m, Ar***H***), 4.88 (1H, dd, *J* = 8.0, 3.0 Hz, 2-***H***), 3.42 – 3.32 (2H, m, 5-***H***$_2$), 2.25 – 2.16 (1H, m, 3-***H***H′), 2.10 – 2.01 (1H, m, 3-H***H***′), 2.00 – 1.91 (1H, m, 4-***H***H′), 1.89 – 1.81 (1H, m, 4-H***H***′), 1.23 (9H, s, C(C***H***$_3$)$_3$); δ_C (176 MHz, $CDCl_3$, mixture of rotamers and diastereomers) 154.2 (***C***=O), 150.7 (Ar***C***), 149.1 (Ar***C***), 137.8 (Ar***C***), 136.9 (Ar***C***), 134.6 (Ar***C***), 129.3 (Ar***C***), 129.0 (Ar***C***), 127.9 (Ar***C***), 122.6 (Ar***C***), 120.9 (Ar***C***), 79.5 (***C***(CH$_3$)$_3$ rotamer A), 79.3 (***C***(CH$_3$)$_3$ rotamer B), 67.3 (***C***-1′′) 53.6 (***C***-2 rotamer A), 52.7 (***C***-2 rotamer B), 46.6 (***C***-5 rotamer B), 46.4 (***C***-5 rotamer A), 32.8 (***C***-3), 28.3 (C(***C***H$_3$)$_3$), 24.6 (***C***-4 rotamer B), 23.4 (***C***-4 rotamer A); *m/z* (LCMS, ESI^+) 439 (M(^{35}Cl)H^+), 441 (M(^{37}Cl)H^+); Accurate mass: Found MH^+, 439.1898: $C_{24}H_{28}N_4O_2{}^{35}Cl$ requires *M*, 439.1901.

(*N*-*tert*-Butoxycarbonyl)-2-(1′-[{4‴-chlorophenyl}{phenyl}methyl]-1′,2′,3′-triazol-4′-yl)pyrrolidine 87B

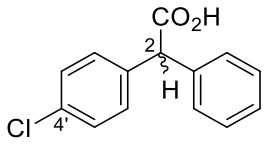
A mixture of (2*R*,1″*R*) and (2*R*,1″*S*) diastereomers



Procedure:[238] A microwave vial was charged with azide **84** (307 mg, 1.26 mmol), alkyne **(*R*)-86** (200 mg, 1.02 mmol), $CuSO_4{\cdot}5H_2O_{(aq.)}$ (1 M, 0.41 mL, 0.41 mmol), freshly prepared Na ascorbate$_{(aq.)}$ (1 M, 2.05 mL, 2.05 mmol), water (1.25 mL) and MeOH (1.25 mL). The vial was sealed and reacted at 60 °C for 30 min, with a 30 s pre-mixing time. The mixture was then cooled, diluted with water (5 mL) and extracted with DCM (3 × 15 mL) and the combined organic extracts washed with brine (25 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The crude residue was then purified by column chromatography (50% → 70% ether in petrol) to provide the *title compound* (432 mg, 96%) as a thick yellow oil.

R_f 0.10 (50% ether in petrol); ν_{max} (ATR) 2978 (w), 2875 (w), 1682 (s), 1491 (m), 1453 (w), 1391 (s), 1364 (m), 1241 (w), 1159 (m), 1109 (w) cm^{-1}; δ_H (400 MHz, $CDCl_3$, mixture of rotamers and diastereomers) 7.42 – 7.26 (5H, m, Ar***H***), 7.17 – 6.96 (6H, m, Ar***H***, 1″-***H***), 4.99 – 4.89 (1H, m, 2-***H***), 3.50 – 3.30 (2H, m, 5-***H***$_2$), 2.48 – 2.38 (0.4H, m, 3-***H***H′ rotamer B), 2.30 – 2.08 (2H, m, 3-***H***$_2$ rotamer A, 3-H***H***′ rotamer B, 4-***H***H′ rotamer B), 2.01 – 1.80 (1.6H, m, 4-***H***$_2$ rotamer A, 4-H***H***′ rotamer B), 1.37 (3.6H, s, C(C***H***$_3$)$_3$ rotamer B), 1.20 (5.4H, s, C(C***H***$_3$)$_3$ rotamer A); δ_C (101 MHz, $CDCl_3$, mixture of rotamers and diastereomers) 154.2 (***C***=O), 150.7 (Ar***C***), 149.1 (Ar***C***), 137.8 (Ar***C***), 137.0 (Ar***C***), 134.5 (Ar***C***), 129.3 (Ar***C***), 129.1 (Ar***C***), 128.0 (Ar***C***), 122.7 (Ar***C***), 121.0 (Ar***C***), 79.4 (***C***(CH$_3$)$_3$ rotamer B), 77.4 (***C***(CH$_3$)$_3$ rotamer A), 67.3 (***C***-1″), 53.6 (***C***-2 rotamer B), 52.8 (***C***-2 rotamer A), 46.4 (***C***-5), 32.8 (***C***-3 rotamer B), 30.7 (***C***-3 rotamer A), 28.3 (C(***C***H$_3$)$_3$), 24.6 (***C***-4 rotamer B), 23.5 (***C***-4 rotamer A); *m/z* (LCMS, ESI$^+$) 439 (M(^{35}Cl)H$^+$), 441 (M(^{37}Cl)H$^+$); Accurate mass: Found MH$^+$, 439.1899: $C_{24}H_{28}N_4O_2{}^{35}Cl$ requires *M*, 439.1895.

(4'-Chlorophenyl)phenylacetic acid[286] **90**



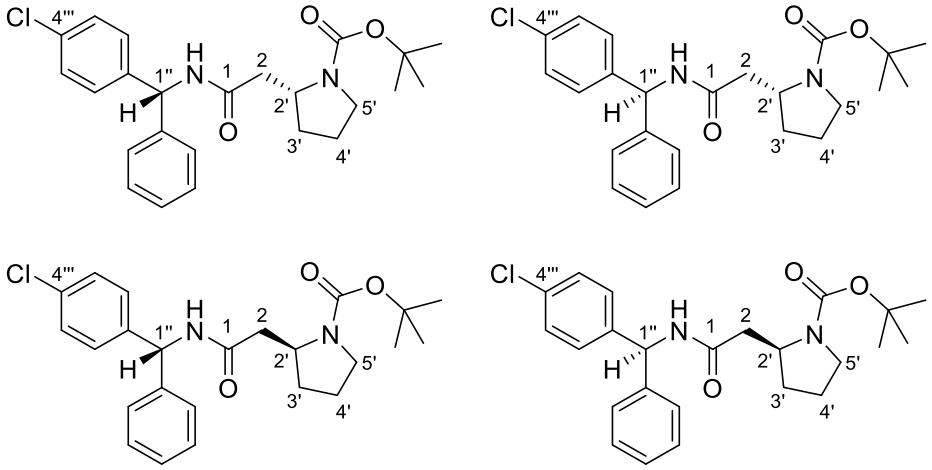
Procedure A:[244] To alcohol **73** (821 mg, 3.75 mmol) at 0 °C was added concentrated H_2SO_4 (33 mL) and formic acid (1.5 mL). The reaction mixture was allowed to reach RT with no stirring, and after 1 h TLC analysis showed consumption of the starting material. Once cooled in an ice bath, H_2O (30 mL) was added and the solution extracted with DCM (3 × 20 mL). The combined organic extracts were washed with 3 M $NaOH_{(aq.)}$ (3 × 20 mL), the aqueous extracts then being acidified and extracted with DCM (3 × 20 mL). Concentration of the organic washings under reduced pressure afforded the title compound (302 mg, 33%) as an off-white solid.

Procedure B:[245] $SnCl_4$ (1.89 mL, 16.00 mmol) was added slowly to a solution of mandelic acid **93** (1.54 g, 10.00 mmol) in chlorobenzene (6 mL). The reaction mixture was heated to reflux for 5.5 h before being cooled in an ice bath and quenched by the slow addition of saturated $NH_4Cl_{(aq.)}$ (20 mL) and H_2O (10 mL). Extraction of the biphasic mixture with DCM (3 × 15 mL) followed by removal of volatiles under reduced pressure provided the crude product which was then recrystallised from acetone and petrol to provide the title compound (985 mg, 40%) as an off-white solid.

R_f 0.52 (10% MeOH in DCM); M.p. 109 – 111 °C (lit.:[287] 116 °C); ν_{max} (ATR) 3028 (br), 1707 (s), 1491 (m), 1403 (w), 1277 (w), 1215 (w), 1092 (w), 1015 (w) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 7.37 – 7.26 (9H, m, Ar***H***), 5.02 (1H, s, 2-***H***); δ_C (176 MHz, $CDCl_3$) 178.2 (***C***=O), 137.6 (Ar***C***), 136.5 (Ar***C***), 133.7 (***C***-4'), 130.2 (Ar***C***), 128.9(6) (Ar***C***), 128.9(5), (Ar***C***), 128.7 (Ar***C***), 127.9 (Ar***C***), 56.4 (***C***-2); *m/z* (LCMS, ESI^-) 201 ($M(^{35}Cl) - CO_2$), 203 ($M(^{37}Cl) - CO_2$).

***N*-([4‴-Chlorophenyl]phenylmethyl)-2-(1′-[*tert*-butoxycarbonyl]pyrrolidin-2′-yl)acetamide 94**

A mixture of (2′*R*,1″*R*), (2′*R*,1″*S*), (2′*S*,1″*R*) and (2′*S*,1″*S*) diastereomers



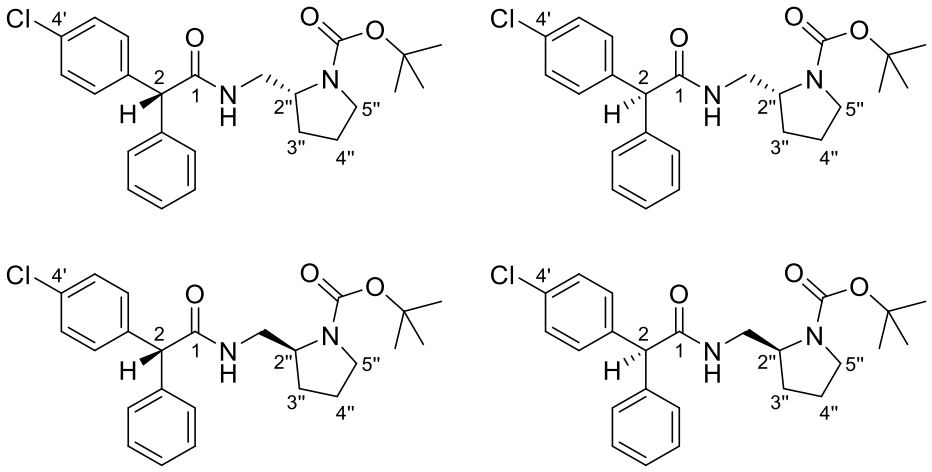
Procedure: Homoproline **62** (57 mg, 0.25 mmol) and 4-chlorobenzhydryl amine HCl salt **89** (98%, 73 mg, 0.28 mmol) were reacted according to general procedure A. SiO_2 column chromatography (0% → 40% EtOAc in hexanes) yielded the *title compound* (72 mg, 67%) as a clear colourless gum.

R_f 0.32 (40% EtOAc in hexanes); ν_{max} (ATR) 3272 (br), 2970 (w), 2922 (w), 2872 (w), 1689 (s), 1642 (s), 1537 (m), 1490 (m), 1394 (s), 1366 (m), 1169 (m), 1124 (m) cm^{-1}; δ_H (700 MHz, $CDCl_3$, mixture of rotamers and diastereomers) 7.33 – 7.30 (2H, m, Ar***H***), 7.29 – 7.25 (3H, m, Ar***H***), 7.23 – 7.16 (4H, m, Ar***H***), 6.20 (1H, d, *J* = 7.5 Hz, 1′′-***H***), 4.08 (1H, tt, *J* = 7.5, 4.0 Hz, 2′-***H***), 3.35 (1H, apparent ddt, *J* = 15.0, 11.0, 7.5 Hz, 5′-***H***H′), 3.27 (1H, apparent dtd, *J* = 11.0, 7.5, 5.0 Hz, 5′-H***H***′), 2.73 (1H, apparent ddd, *J* = 14.0, 9.5, 4.0 Hz, 2-***H***H′), 2.56 – 2.41 (1H, m, 2-H***H***′), 2.03 – 1.97 (1H, m, 3′-***H***H′), 1.97 – 1.90 (1H, m, 3′-H***H***′), 1.88 – 1.82 (1H, m, 4′-***H***H′), 1.82 – 1.76 (1H, m, 4′-H***H***′), 1.43 – 1.41 (9H, m, C(C***H***$_3$)$_3$); δ_C (176 MHz, $CDCl_3$, mixture of rotamers and diastereomers) 170.3 (***C***-1), 155.2 (***C***(O)NO), 141.3 (Ar***C***), 141.2 (Ar***C***), 140.5 (Ar***C***), 140.3 (Ar***C***), 133.3 (Ar***C***), 133.2 (Ar***C***), 128.9(0) (Ar***C***), 128.8(7) (Ar***C***), 128.8(4) (Ar***C***), 128.8(2) (Ar***C***), 127.8 (Ar***C***), 127.7 (Ar***C***), 127.5(8) (Ar***C***), 127.5(6) (Ar***C***), 80.0(1) (***C***(CH$_3$)$_3$), 79.9(8) (***C***(CH$_3$)$_3$), 56.5(7) (***C***-1′′), 56.5(5) (***C***-1′′), 55.3 (***C***-2′), 55.2 (***C***-2′), 46.9 (***C***-5′), 46.8 (***C***-5′), 41.8(2) (***C***-2), 41.7(6) (***C***-2),

31.3(7) (***C***-3′), 31.3(5) (***C***-3′), 28.6 (C(***C***H$_3$)$_3$), 23.6 (***C***-4′); *m/z* (LCMS, ESI$^+$) 429 (M(^{35}Cl)H$^+$), 431 (M(^{37}Cl)H$^+$); Accurate mass: Found MH$^+$, 429.1939: $C_{24}H_{30}N_2O_3{}^{35}Cl$ requires *M*, 429.1945.

_N_-([1″-{*tert*-Butoxycarbonyl}pyrrolidin-2″-yl]methyl)-2-(4′-chlorophenyl)-2-phenylacetamide 95

A mixture of (2″*R*,2*R*), (2″*R*,2*S*), (2″*S*,2*R*) and (2″*S*,2*S*) diastereomers



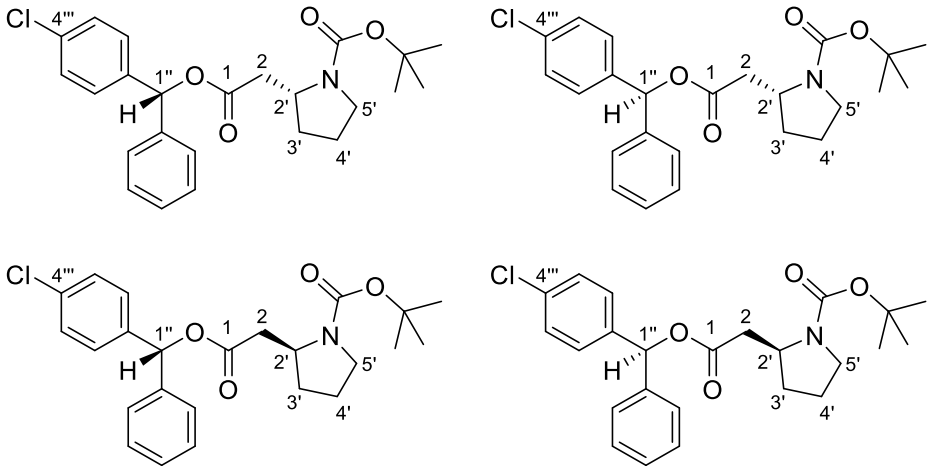
Procedure: Amine **91** (100 mg, 0.53 mmol) and acid **90** (131 mg, 0.53 mmol) were reacted according to general procedure A. SiO_2 column chromatography (0% → 50% EtOAc in hexanes) yielded the *title compound* (132 mg, 58%) as a viscous clear colourless oil.

R_f 0.31 (40% EtOAc in hexanes); ν_{max} (ATR) 3298 (br), 3058 (w), 2968 (w), 2938 (w), 2876 (w), 1653 (br, s), 1538 (m), 1486 (m), 1388 (s), 1365 (m), 1165 (s), 1104 (m) cm^{-1}; δ_H (700 MHz, $CDCl_3$, mixture of rotamers and diastereomers) 7.37 – 7.18 (9H, m, Ar***H***), 4.82 – 4.80 (1H, m, 2-***H***), 3.98 – 3.88 (1H, m, 2′′-***H***), 3.48 – 3.40 (1H, m, NH(C***H***H′)), 3.35 (1H, dt, *J* = 11.0, 7.5 Hz, 5′′-***H***H′), 3.29 – 3.16 (2H, m, 5′′-H***H***′, NH(CH***H***′)), 2.00 – 1.91 (1H, m, 3′′-***H***H′), 1.88 – 1.74 (2H, m, 4′′-***H***$_2$), 1.64 (1H, apparent ddd, *J* = 16.5, 7.0, 4.0 Hz, 3′′-H***H***′), 1.44 – 1.37 (9H, m, C(C***H***$_3$)$_3$); δ_C (176 MHz, $CDCl_3$, mixture of rotamers and diastereomers) 171.8(4) (***C***-1), 171.8(2) (***C***-1), 156.5 (***C***(O)NO), 139.4 (Ar***C***), 139.3 (Ar***C***), 138.3(4) (Ar***C***), 138.2(7) (Ar***C***), 133.1 (Ar***C***), 133.0 (Ar***C***), 130.3(9) (Ar***C***), 130.3(8) (Ar***C***), 128.9 (Ar***C***), 128.8(0) (Ar***C***), 128.7(7) (Ar***C***), 128.7(6) (Ar***C***), 127.4 (Ar***C***), 80.1(7) (***C***(CH$_3$)$_3$), 80.1(5) (***C***(CH$_3$)$_3$), 58.5 (***C***-2), 56.5 (***C***-2′′), 47.3 (***C***-5′′), 46.4

(NH(***C***HH')), 29.8(0) (***C***-3''), 29.7(7) (***C***-3''), 28.5(2) (C(***C***H_3)$_3$), 28.5(0) (C(***C***H_3)$_3$), 23.9 (***C***-4''); *m/z* (LCMS, ESI^+) 429 ($M(^{35}Cl)H^+$), 431 ($M(^{37}Cl)H^+$); Accurate mass: Found MH^+, 429.1937: $C_{24}H_{30}N_2O_3{}^{35}Cl$ requires *M*, 429.1945.

([4'''-Chlorophenyl]phenylmethyl)-2-(1'-[*tert*-butoxycarbonyl]pyrrolidin-2'-yl)acetate 96

A mixture of (2'*R*,1''*R*), (2'*R*,1''*S*), (2'*S*,1''*R*) and (2'*S*,1''*S*) diastereomers



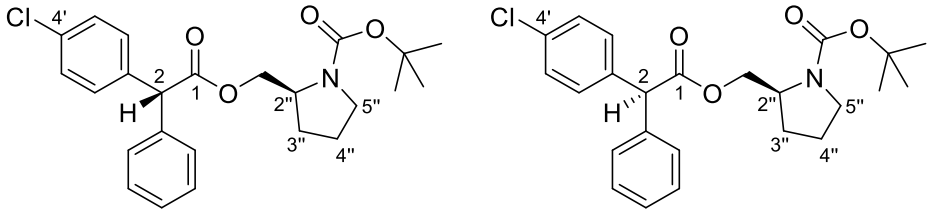
Procedure:[246] Homoproline **62** (57 mg, 0.25 mmol) was dissolved in DCM (1.7 mL) and cooled to 0 °C prior to the addition of alcohol **73** (35 mg, 0.25 mmol), 4-dimethylaminopyridine (35 mg, 0.28 mmol), *N*-methylmorpholine (31 µL, 0.28 mmol) and EDCI·HCl (54 mg, 0.28 mmol). The reaction mixture was stirred at RT for 24 h before being cooled in an ice bath, quenched with ice cold 1 M $HCl_{(aq.)}$ (0.75 mL) and stirred for 5 min. The biphasic mixture was diluted with H_2O (10 mL), extracted with DCM (3 × 5 mL) and the collected organic extracts were washed with saturated $NaHCO_{3(aq.)}$ (2 × 5 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Silica gel chromatography (0% → 40% EtOAc in hexanes) of the crude material furnished the *title compound* (58 mg, 54%) as a clear colourless oil.

R_f 0.47 (30% EtOAc in hexanes); ν_{max} (ATR) 2978 (w), 2930 (w), 2876 (w), 1737 (w), 1690 (s), 1490 (w), 1395 (s), 1252 (w), 1109 (m), 1090 (w) cm^{-1}; δ_H (700 MHz, $CDCl_3$, mixture of rotamers and diastereomers) 7.35 – 7.25 (9H, m, Ar***H***), 6.84 (1H, s, 1''-***H***), 4.19 – 4.11 (1H, m, 2'-***H***), 3.13 – 3.25 (2H, m, 5'-***H***$_2$), 3.13 – 2.88 (1H, m, 2-***H***H'), 2.43 (1H, dd, *J* = 15.0, 10.0 Hz, 2-H***H***'), 2.00 (1H, dq, *J* = 13.0, 8.0 Hz, 3'-***H***H'), 1.82 – 1.75 (2H, m, 4'-***H***$_2$), 1.72 – 1.66 (1H, m, 3'-H***H***'), 1.49 – 1.44 (9H, m, C(C***H***$_3$)$_3$); δ_C (176 MHz, $CDCl_3$, mixture of rotamers and diastereomers) 170.5 (***C***-1),

154.4 (***C***(O)NO), 143.6 (Ar***C***), 142.4 (Ar***C***), 139.8 (Ar***C***), 138.9 (Ar***C***), 133.9 (Ar***C***), 133.4 (Ar***C***), 128.8(3) (Ar***C***), 128.7(8) (Ar***C***), 128.7(3) (Ar***C***), 128.6(6) (Ar***C***), 128.6 (Ar***C***), 128.3 (Ar***C***), 128.0(2) (Ar***C***), 127.9(8) (Ar***C***), 127.2 (Ar***C***), 127.1 (Ar***C***), 126.7 (Ar***C***), 79.7 (***C***$(CH_3)_3$), 76.3 (Ar_2***C***H), 75.8 (***C***-1''), 54.2 (***C***-2'), 46.6 (***C***-5'), 39.7 (***C***-2), 38.8 (***C***-2), 31.0 (***C***-3'), 28.7 (C(***C***$H_3)_3$), 23.6 (***C***-4'); *m/z* (LCMS, ESI^+) 452 ($M(^{35}Cl)Na^+$), 454 ($M(^{37}Cl)Na^+$); Accurate mass: Found MNa^+, 452.1606: $C_{24}H_{28}NO_4{}^{35}Cl^{23}Na$ requires *M*, 452.1605.

([1''-{*tert*-Butoxycarbonyl}pyrrolidin-2''-yl]methyl)-2-(4'-chlorophenyl)-2-phenylacetic acid 97

A mixture of (2''*S*,2*R*) and (2''*S*,2*S*) diastereomers

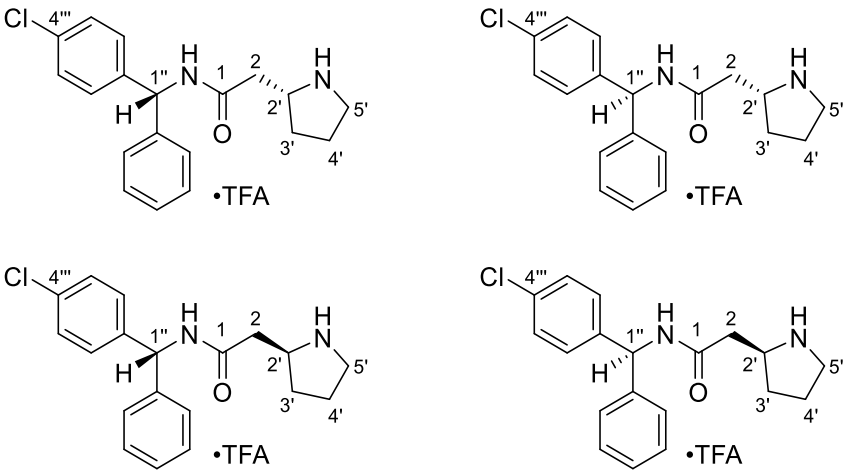


Procedure:[246] Prolinol **(*S*)-59** (101 mg, 0.50 mmol) and acid **90** (123 mg, 0.50 mmol) were subjected to the esterification conditions utilised in the synthesis of ester **96** to give the *title compound* (152 mg, 71%) as a clear oil.

R_f 0.45 (30% EtOAc in hexanes); ν_{max} (ATR) 2973 (w), 2876 (w), 1735 (m), 1690 (s), 1450 (m), 1389 (s), 1134 (m), 1090 (m), 1014 (m) cm^{-1}; δ_H (700 MHz, $CDCl_3$, mixture of rotamers and diastereomers) 7.38 – 7.14 (9H, m, Ar***H***), 4.98 (1H, s, 2-***H***), 4.31 – 4.13 (2H, m, OCH_2), 4.06 – 3.96 (0.5H, m, 2''-***H*** isomer 1), 3.94 – 3.84 (0.5H, m, 2''-***H*** isomer 2), 3.37 – 3.08 (2H, m, 5''-H_2), 1.89 – 1.79 (1H, m, 3''-***H***H'), 1.72 – 1.59 (3H, m, 3''-H***H***', 4''-H_2), 1.44 (9H, s, C(C$H_3)_3$); δ_C (176 MHz, $CDCl_3$, mixture of rotamers and diastereomers) 171.9(***C***-1), 154.5 (***C***(O)NO), 154.3 (***C***(O)NO), 138.3 (Ar***C***), 138.1 (Ar***C***), 137.3 (Ar***C***), 137.1 (Ar***C***), 133.3 (Ar***C***), 133.2 (Ar***C***), 130.1 (Ar***C***), 128.8 (Ar***C***), 128.5 (Ar***C***), 127.6 (Ar***C***), 79.8 (***C***$(CH_3)_3$), 79.5 (***C***$(CH_3)_3$), 65.7 (O***C***H_2), 65.3 (O***C***H_2), 56.5(9) (***C***-2), 56.5(8) (***C***-2), 55.6 (***C***-2''), 55.5 (***C***-2''), 46.8 (***C***-5''), 46.6 (***C***-5''), 29.0 (***C***-3''), 28.5 (C(***C***$H_3)_3$), 28.0 (***C***-3''), 23.8 (***C***-4''), 23.0 (***C***-4''); *m/z* (LCMS, ESI^+) 430 ($M(^{35}Cl)H^+$), 432 ($M(^{37}Cl)H^+$); Accurate mass: Found MH^+, 430.1774: $C_{24}H_{29}NO_4{}^{35}Cl$ requires *M*, 430.1785.

***N*-([4‴-Chlorophenyl]phenylmethyl)-2-(pyrrolidin-2′-yl)acetamide TFA salt 98**

A mixture of (2′*R*,1″*R*), (2′*R*,1″*S*), (2′*S*,1″*R*) and (2′*S*,1″*S*) diastereomers

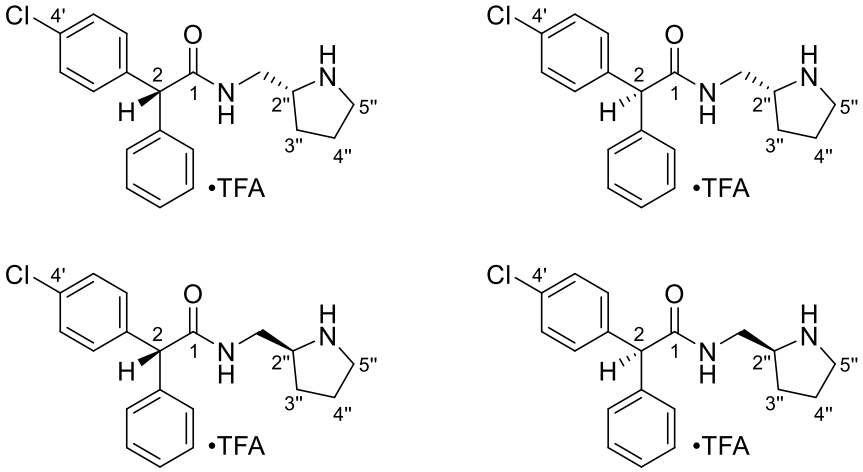


Procedure: Amide **94** (56 mg, 0.13 mmol) was dissolved in a mixture of DCM (0.9 mL) and TFA (0.1 mL) and stirred for 3 h, after which all volatiles were removed under high vacuum to provide the *title compound* (58 mg, 77%) as an off-white solid.

M.p. 189 – 191 °C; ν_{max} (ATR) 3262 (br), 3031 (w), 2951 (w), 2360 (w), 1653 (br, s), 1542 (m), 1491 (m), 1197 (s), 1137 (s) cm^{-1}; δ_H (600 MHz, $CDCl_3$, mixture of diastereomers) 9.17 – 9.06 (1H, m, 1′-N***H***$_2^+$), 8.92 – 8.82 (1H, m, 1′-N***H***$_2^+$), 7.52 (0.5H, d, *J* = 7.0 Hz, 1-N***H*** isomer 1), 7.47 (0.5H, d, *J* = 7.0 Hz, 1-N***H*** isomer 2), 7.34 – 7.24 (5H, m, Ar***H***), 7.20 – 7.12 (4H, m, Ar***H***), 6.03 – 5.97 (1H, m, 1′′-***H***), 3.79 – 3.71 (1H, m, 2′-***H***), 3.21 – 3.13 (1H, m, 5′-***H***H′), 3.13 – 3.05 (1H, m, 5′-H***H***′), 3.01 – 2.94 (1H, m, 2-***H***H′), 2.77 – 2.69 (1H, m, 2-H***H***′), 2.12 – 2.04 (1H, m, 3′-***H***H′), 2.02 – 1.94 (1H, m, 4′-***H***H′), 1.91 – 1.82 (1H, m, 4′-H***H***′), 1.70 – 1.62 (1H, m, 3′-H***H***′); δ_C (151 MHz, $CDCl_3$, mixture of diastereomers) 170.0(4) (***C***-1), 170.0(2) (***C***-1), 161.3 (q, *J* = 38.5 Hz, TFA ***C***=O), 140.3(2) (Ar***C***), 140.3(1) (Ar***C***), 139.4 (Ar***C***), 133.8 (Ar***C***), 133.6 (Ar***C***), 129.1 (Ar***C***), 129.0(3) (Ar***C***), 129.0(0) (Ar***C***), 128.9 (Ar***C***), 128.7 (Ar***C***), 128.2 (Ar***C***), 128.0 (Ar***C***), 127.5 (Ar***C***), 127.2 (Ar***C***), 57.6 (***C***-1′′), 57.5 (***C***-1′′), 57.2 (***C***-2′), 57.1 (***C***-2′), 45.5 (***C***-5′), 36.3(6) (***C***-2), 36.3(5) (***C***-2), 30.0 (***C***-3′), 29.9 (***C***-3′), 23.7(7) (***C***-4′), 23.7(5) (***C***-4′); δ_F (376 MHz, $CDCl_3$) −76.19 (C***F***$_3$CO$_2$H); *m/z* (LCMS, ESI^+) 329 (M(^{35}Cl)H^+), 331 (M(^{37}Cl)H^+); Accurate mass: Found MH^+, 329.1422: $C_{19}H_{22}N_2O^{35}Cl$ requires *M*, 329.1421.

***N*-([Pyrrolidin-2′′-yl]methyl)-2-(4′-chlorophenyl)-2-phenylacetamide TFA salt 99**

A mixture of (2′′*R*,2*R*), (2′′*R*,2*S*), (2′′*S*,2*R*) and (2′′*S*,2*S*) diastereomers

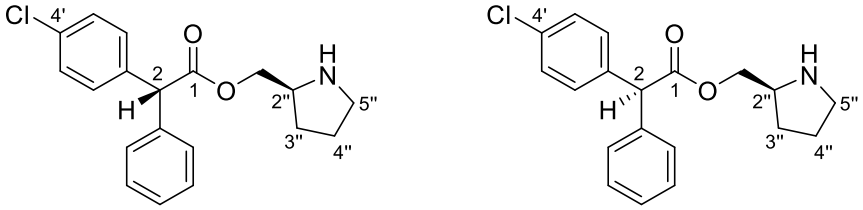


Procedure: The protected amide **95** was subjected to the same conditions used for the synthesis of amide **98**, with the crude material being recrystallised from EtOAc and ether to give the *title compound* (38 mg, 28%) as a white solid.

M.p. 149 – 151 °C; ν_{max} (ATR) 3252 (br), 3066 (w), 1665 (s), 1646 (s), 1563 (m), 1191 (m), 1156 (m) 1133 (s) cm^{-1}; δ_H (600 MHz, $CDCl_3$, mixture of diastereomers) 10.13 – 9.95 (1H, m, 1′′-N***H***$_2^+$), 8.85 – 8.65 (1H, m, 1′′-N***H***$_2^+$), 8.06 (0.5H, t, *J* = 6.0 Hz, 1-N***H*** isomer 1), 7.98 (0.5H, t, *J* = 6.0 Hz, 1-N***H*** isomer 2), 7.40 – 7.10 (9H, m, Ar***H***), 4.92 (1H, s, 2-***H***), 3.76 – 3.63 (1H, m, 2′′-***H***), 3.62 – 3.50 (1H, m, NH(C***H***H′)), 3.45 – 3.29 (1H, m, NH(CH***H***′)), 3.13 – 2.94 (2H, m, 5′′-***H***$_2$), 2.02 – 1.93 (1H, m, 3′′-***H***H′), 1.92 – 1.80 (2H, m, 4′′-***H***$_2$), 1.65 – 1.52 (1H, m, 3′′-H***H***′); δ_C (151 MHz, $CDCl_3$, mixture of diastereomers) 174.4 (9) (***C***-1), 174.4(8) (***C***-1), 162.3 (q, *J* = 37.5 Hz, TFA ***C***=O), 138.8 (Ar***C***), 138.6 (Ar***C***), 137.7 (Ar***C***), 137.5 (Ar***C***), 133.4(3) (Ar***C***), 133.3(9) (Ar***C***), 130.3(4) (Ar***C***), 130.2(7) (Ar***C***), 129.0 (Ar***C***), 128.9 (Ar***C***), 128.8 (Ar***C***), 128.7 (Ar***C***), 127.7(0) (Ar***C***), 127.6(6) (Ar***C***), 60.7(1) (***C***-2′′), 60.6(9) (***C***-2′′), 57.4(3) (***C***-2), 57.3(8) (***C***-2), 45.4 (***C***-5′′), 45.3 (***C***-5′′), 40.8 (NH(***C***HH′)), 27.6 (***C***-3′′), 24.0 (***C***-4′′); δ_F (376 MHz, $CDCl_3$) −75.88 (C***F***$_3$$CO_2$H); *m/z* (LCMS, ESI^+) 329 (M(^{35}Cl)H^+), 331 (M(^{37}Cl)H^+); Accurate mass: Found MH^+, 329.1426: $C_{19}H_{22}N_2O^{35}Cl$ requires *M*, 329.1421.

([Pyrrolidin-2''-yl]methyl)-2-(4'-chlorophenyl)-2-phenylacetic acid 100

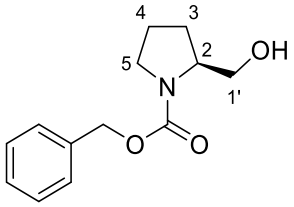
A mixture of (2''*S*,2*R*) and (2''*S*,2*S*) diastereomers



Procedure: Ester **97** was deprotected using the same procedure for the synthesis of salt **98**, with the resulting crude material being neutralised with 3 M $NaOH_{(aq.)}$ (2 mL) and extracted with EtOAc (3 × 5 mL). The collected organic extracts were dried over $MgSO_4$ and concentrated *in vacuo* before being purified by preparative TLC (5% MeOH and 1% $NH_4OH_{(aq.)}$ in DCM) to yield the *title compound* (17 mg, 15%) as a clear colourless oil.

R_f 0.83 (10% MeOH and 1% $NH_4OH_{(aq.)}$ in $CHCl_3$); ν_{max} (ATR) 3364 (br), 2959 (m), 2877 (m), 1620 (s), 1489 (m), 1421 (s), 1263 (w), 1174 (w), 1087 (m), 1048 (m), 1015 (m) cm^{-1}; δ_H (700 MHz, $CDCl_3$, mixture of diastereomers) 7.37 – 7.15 (9H, m, Ar***H***), 5.08 – 5.05 (1H, m, 2-***H***), 4.31 – 4.27 (1H, m, 2''-***H***), 3.68 (1H, apparent ddd, *J* = 11.5, 5.0, 2.5 Hz, O(C***H***H')), 3.64 – 3.57 (1.5H, m, 5''-***H***H' isomer 1, O(CH***H***')), 3.54 (0.5H, dt, *J* = 10.0, 7.0 Hz, 5''-***H***H' isomer 2), 3.44 (0.5H, dt, *J* = 10.0, 7.0 Hz, 5''-H***H***' isomer 2), 3.35 (0.5H, dt, *J* = 10.5, 7.0 Hz, 5''-H***H***' isomer 1), 2.03 (1H, apparent ddt, *J* = 20.0, 14.5, 7.5 Hz, 3''-***H***H'), 1.92 – 1.84 (1H, m, 4''-***H***H'), 1.84 – 1.72 (1H, m, 4''-H***H***'), 1.61 – 1.55 (1H, m, 3''-H***H***'); δ_C (176 MHz, $CDCl_3$, mixture of diastereomers) 172.8(9) (***C***-1), 172.8(8) (***C***-1), 138.6 (Ar***C***), 138.3 (Ar***C***), 137.8 (Ar***C***), 137.6 (Ar***C***), 133.3(1) (Ar***C***), 133.2(7) (Ar***C***), 130.6 (Ar***C***), 130.5 (Ar***C***), 129.1 (Ar***C***), 129.0 (Ar***C***), 128.9 (Ar***C***), 128.8 (Ar***C***), 127.6(1) (Ar***C***), 127.5(7) (Ar***C***), 67.6 (O(***C***HH')), 65.5 (O(***C***HH')), 62.0(1) (***C***-2''), 61.9(8) (***C***-2''), 56.2(2) (***C***-2), 56.2(0) (***C***-2), 48.3(7) (***C***-5''), 48.3(5) (***C***-5''), 28.5 (***C***-3''), 28.4 (***C***-3''), 24.6 (***C***-4''), 24.5 (***C***-4''); *m/z* (LCMS, ESI^+) 330 ($M(^{35}Cl)H^+$), 332 ($M(^{37}Cl)H^+$); Accurate mass: Found MH^+, 330.1273: $C_{19}H_{21}NO_2{}^{35}Cl$ requires *M*, 330.1261.

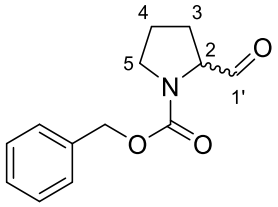
(2*S*)-(*N*-Carboxybenzyl)-2-(hydroxymethyl)pyrrolidine[288] (*S*)-104



Procedure:[249] A round bottomed flask equipped with a reflux condenser was charged with (*S*)-*N*-Z-proline **(*S*)-68** (1.65 g, 6.62 mmol) and THF (33 mL). The mixture was cooled in an ice bath before dropwise addition of a solution of $BH_3{\cdot}SMe_2$ (0.69 mL, 7.28 mmol) in THF (3 mL). The reaction mixture was heated to reflux for 2 h, after which complete conversion of starting material was observed by TLC. After cooling to 0 °C, the reaction was quenched with the addition of cold water (5 mL) and THF was removed from the crude mixture under reduced pressure. The residual material was diluted with water (30 mL) and extracted with DCM (3 × 20 mL). The combined organic extracts were washed with brine (30 mL) and dried over $MgSO_4$ before being concentrated under reduced pressure. Column chromatography (10% → 50% EtOAc in hexanes) furnished the title compound (1.17 g, 75%) as a viscous clear colourless oil.

R_f 0.21 (50% EtOAc in hexanes); $[\alpha]^{28}_D$ (c = 1.00 g/100 mL, $CHCl_3$) −40.43° (lit.:[289] $[\alpha]^{20}_D$ (c = 1.00 g/100 mL, $CHCl_3$) −40.2°); ν_{max} (ATR) 3391 (br), 2950 (w), 2876 (w), 1670 (s), 1414 (s), 1356 (s), 1191 (m), 1103 (s), 1048 (m) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 7.38 – 7.34 (4H, m, Ar***H***), 7.34 – 7.30 (1H, m, Ar***H***), 5.17 – 5.11 (2H, m, PhC***H***$_2$), 4.05 – 3.91 (1H, m, 2-***H***), 3.70 – 3.66 (1H, m, 1'-***H***H'), 3.63 (1H, dd, *J* = 11.5, 7.5 Hz, 1'-H***H***'), 3.55 (1H, dt, *J* = 11.5, 7.0 Hz, 5-***H***H'), 3.39 (1H, dt, *J* = 10.5, 7.0 Hz, 5-H***H***'), 2.06 – 1.98 (1H, m, 3-***H***H'), 1.92 – 1.83 (1H, m, 4-***H***H'), 1.83 – 1.76 (1H, m, 4-H***H***'), 1.60 (1H, dq, *J* = 13.5, 7.0 Hz, 3-H***H***'); δ_C (176 MHz, $CDCl_3$) 157.3 (N***C***O_2), 136.6 (Ar***C***), 128.6 (Ar***C***), 128.2 (Ar***C***), 128.0 (Ar***C***), 67.4 (Ph***C***H_2), 67.2 (***C***-1'), 60.9 (***C***-2), 47.5 (***C***-5), 28.8 (***C***-3), 24.2 (***C***-4); *m/z* (LCMS, ESI^+) 236 (MH^+), 258 (MNa^+).

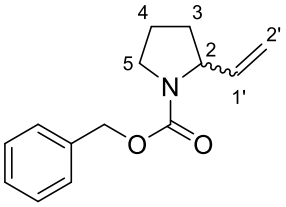
(*N*-Carboxybenzyl)-2-formylpyrrolidine[290] 105



Procedure:[250] To a flask fitted with a condenser was added alcohol **(*S*)-104** (1.25 g, 5.31 mmol), $Cu(MeCN)_4PF_6$ (101 mg, 0.27 mmol), 2,2′-bipyridine (42 mg, 0.27 mmol), TEMPO (43 mg, 0.27 mmol), *N*-methylimidazole (43 µL, 0.53 mmol) and MeCN (25 mL). The reaction mixture was stirred, open to air, at 70 °C for 18 h after which TLC analysis showed consumption of the starting material. A 1:1 mixture of hexanes and ether (20 mL) was added and the solids removed by filtration through a celite® plug before being washed with a small amount of 1:1 hexanes : ether. The collected filtrates were concentrated *in vacuo* before purification by SiO_2 column (0% → 30% EtOAc in hexanes) provided the title compound (520 mg, 42%) as a clear oil.

R_f 0.35 (25% EtOAc in hexanes); ν_{max} (ATR) 2946 (w), 2882 (w), 1786 (w), 1696 (s, br), 1406 (s), 1352 (m), 1294 (m), 1110 (m), 1039 (m) cm^{-1}; δ_H (700 MHz, $CDCl_3$, mixture of rotamers) 9.60 – 9.58 (0.5H, br m, 1'-***H*** rotamer A), 9.49 (0.5H, d, *J* = 2.0 Hz, 1'-***H*** rotamer B), 7.39 – 7.28 (5H, m, Ar***H***), 5.20 – 5.10 (2H, m, PhC$\boldsymbol{H}_2$), 4.31 – 4.28 (0.5H, m, 2-***H*** rotamer A), 4.22 – 4.18 (0.5H, m, 2-***H*** rotamer B), 3.63 – 3.49 (2H, m, 5-$\boldsymbol{H}_2$), 2.13 (0.5H, dq, *J* = 13.5, 8.0 Hz, 3-***H***H' rotamer A), 2.10 – 2.04 (0.5H, m, 3-***H***H' rotamer B), 2.04 – 1.99 (1H, m, 3-H***H***' rotamer A, 3-H***H***' rotamer B), 1.95 – 1.81 (2H, m, 4-$\boldsymbol{H}_2$); δ_C (176 MHz, $CDCl_3$, mixture of rotamers) 200.1 (***C***-1'), 155.5 (N***C***O_2), 154.6 (N***C***O_2), 136.6 (Ar***C***), 136.4 (Ar***C***), 128.6 (Ar***C***), 128.2 (Ar***C***), 128.1 (Ar***C***), 67.4(2) (Ph***C***H_2), 67.3(7) (Ph***C***H_2), 65.4 (***C***-2 rotamer A), 65.0 (***C***-2 rotamer B), 47.4 (***C***-5), 46.8 (***C***-5), 28.0 (***C***-3 rotamer A), 26.7 (***C***-3 rotamer B), 24.6 (***C***-4), 23.9 (***C***-4); *m/z* (LCMS, ESI^+) 234 (MH^+), 256 (MNa^+).

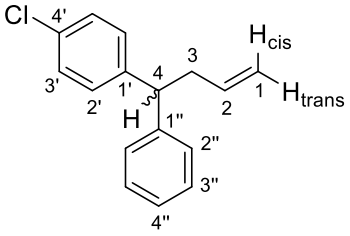
(*N*-Carboxybenzyl)-2-vinylpyrrolidine[291] 106



Procedure:[229] Methyltriphenylphosphonium bromide (1.04 g, 2.85 mmol) was dissolved in THF (6 mL) and cooled to −10 °C. Butyllithium (1.6 M in hexanes, 1.78 mL, 2.85 mmol) was added slowly. The resultant dark orange solution was stirred at RT for 30 min then re-cooled prior to addition of aldehyde **105** (398 mg, 1.71 mmol) in THF (2 mL). The resultant dark red solution was reacted at RT for 2.5 h then quenched by addition of saturated $NH_4Cl_{(aq.)}$ (10 mL) before removal of volatiles under reduced pressure. The crude residue was diluted with H_2O (20 mL) and extracted with ether (3 × 15 mL). The combined organic extracts were washed with brine (20 mL) and saturated $NaHCO_{3(aq.)}$ (20 mL) before being dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography (0% → 25% EtOAc in hexanes) yielded the title compound (214 mg, 54%) as a thin clear colourless oil.

R_f 0.34 (25% EtOAc in hexanes); ν_{max} (ATR) 2974 (w), 2928 (w), 2884 (w), 1698 (s), 1409 (s), 1352 (m), 1094 (m) cm^{-1}; δ_H (700 MHz, $CDCl_3$, mixture of rotamers) 7.40 – 7.28 (5H, m, Ar***H***), 5.82 – 5.71 (1H, m, 1′-***H***), 5.19 – 5.08 (3H, m, PhC***H***$_2$, 2′-***H***H′), 5.08 – 4.99 (1H, m, 2′-H***H***′), 4.45 – 4.36 (1H, m, 2-***H***), 3.52 – 3.41 (2H, m, 5-***H***$_2$), 2.06 – 1.97 (1H, m, 3-***H***H′), 1.92 – 1.81 (2H, m, 4-***H***$_2$), 1.77 – 1.71 (1H, m, 3-H***H***′); δ_C (176 MHz, $CDCl_3$, mixture of rotamers) 155.0 (N***C***O_2), 138.7 (***C***-1′), 138.2 (***C***-1′), 137.2 (Ar***C***), 128.6 (Ar***C***), 128.5 (Ar***C***), 128.0 (Ar***C***), 127.9 (Ar***C***), 114.5 (***C***-2′), 114.2 (***C***-2′), 66.8 (Ph***C***H_2), 59.6 (***C***-2), 59.2 (***C***-2), 46.9 (***C***-5), 46.5 (***C***-5), 32.2 (***C***-3), 31.4 (***C***-3), 23.6 (***C***-4), 22.7 (***C***-4); *m/z* (LCMS, ESI^+) 232 (MH^+).

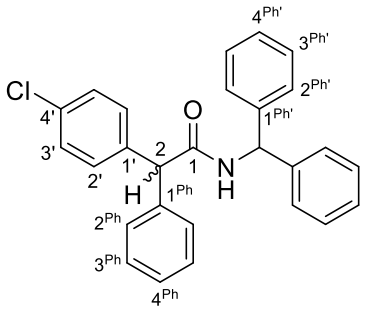
4-(4′-Chlorophenyl)-4-phenylbut-1-ene[292] **107**



Procedure: A flask was charged with alcohol **73** (262 mg, 1.20 mmol), DCM (2.4 mL) and NEt_3 (0.17 mL, 1.20 mmol) before methanesulfonyl chloride (93 µL, 1.20 mmol) was added dropwise at RT. The solution was stirred until TLC analysis showed consumption of alcohol **73**, after which the reaction mixture was cooled to −10 °C and toluene (6 mL) was added followed by the slow addition of allyl grignard reagent (1 M in ether, 3.60 mL, 3.60 mmol) and the reaction allowed to warm slowly to RT overnight. The reaction was cooled in an ice bath, quenched with saturated $NH_4Cl_{(aq.)}$ (10 mL) and extracted with DCM (3 × 10 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated *in vacuo* before being purified by column chromatography (0% → 8% ether in hexanes) to provide the title compound (214 mg, 74%) as a clear colourless oil.

R_f 0.71 (10% ether in hexanes); ν_{max} (ATR) 3070 (w), 3028 (w), 2924 (w), 2844 (w), 1640 (w), 1599 (w), 1489 (s), 1449 (w), 1407 (w), 1262 (w), 1091 (s), 1013 (s) cm^{-1}; δ_H (600 MHz, $CDCl_3$) 7.30 – 7.27 (2H, m, 3′′-***H***$_2$), 7.26 – 7.23 (2H, m, 3′-***H***$_2$), 7.22 – 7.15 (5H, m, 2′-***H***$_2$, 2′′-***H***$_2$, 4′′-***H***), 5.70 (1H, ddt, *J* = 17.0, 10.0, 7.0 Hz, 2-***H***), 5.03 (1H, ddt, *J* = 17.0, 2.0, 1.5 Hz, 1-***H***$_{cis}$), 4.96 (1H, ddt, *J* = 10.0, 2.0, 1.0 Hz, 1-***H***$_{trans}$), 3.99 (1H, t, *J* = 8.0 Hz, 4-***H***), 2.84 – 2.74 (2H, m, 3-***H***$_2$); δ_C (151 MHz, $CDCl_3$) 144.1 (***C***-1′′), 143.1 (***C***-1′), 136.5 (***C***-2), 132.1 (***C***-4′), 129.5 (***C***-2′), 128.7 (***C***-3′, ***C***-3′′), 128.0 (Ar***C***), 126.5 (Ar**C**), 116.8 (***C***-1), 50.7 (***C***-4), 40.0 (***C***-3).

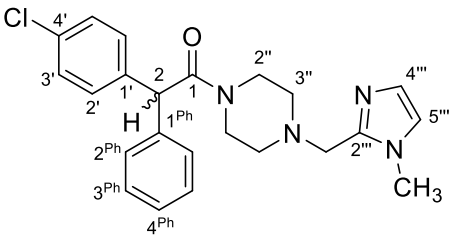
***N*-(Diphenylmethyl)-2-(4′-chlorophenyl)-2-phenylacetamide 110**



Procedure: Acid **90** (50 mg, 0.20 mmol) and benzhydrylamine **112** (36 µL, 0.20 mmol) were reacted according to general procedure A. Following reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (55 mg, 67%) was isolated as an off-white solid.

M.p. 196 – 199 °C; ν_{max} (ATR) 3270 (br), 3070 (w), 3021 (w), 1647 (s), 1490 (m), 1481 (m), 1368 (w), 1222 (w) cm^{-1}; δ_H (600 MHz, $CDCl_3$) 7.35 – 7.26 (11H, m, 3′-***H***$_2$, $3^{Ph'}$-(***H***$_2$)$_2$, $4^{Ph'}$-(***H***)$_2$, 3^{Ph}-***H***$_2$, 4^{Ph}-***H***), 7.22 (2H, d, *J* = 7.5 Hz, 2^{Ph}-***H***$_2$), 7.18 (2H, d, *J* = 8.0 Hz, 2′-***H***$_2$), 7.11 (4H, d, *J* = 8.0 Hz, $2^{Ph'}$-(***H***$_2$)$_2$), 6.29 (1H, d, *J* = 8.0 Hz, NHC***H***Ph$_2$), 6.17 (1H, d, *J* = 8.0 Hz, N***H***CHPh$_2$), 4.93 (1H, s, 2-***H***); δ_C (151 MHz, $CDCl_3$) 170.6 (***C***=O), 141.3(3) (***C***-$1^{Ph'}$), 141.3(0) (***C***-$1^{Ph'}$), 139.0 (***C***-1^{Ph}), 137.9 (***C***-1′), 133.4 (***C***-4′), 130.4 (***C***-2′), 129.1 (Ar***C***), 129.0 (Ar***C***), 128.9 (Ar***C***), 128.8 (Ar***C***), 127.8 (Ar***C***), 127.7 (Ar***C***), 127.4 (***C***-$2^{Ph'}$), 58.5 (***C***-2), 57.3 (NH***C***HPh$_2$); *m/z* (LCMS, ESI^+) 412 ($M(^{35}Cl)H^+$), 414 ($M(^{37}Cl)H^+$); Accurate mass: Found MH^+, 412.1473: $C_{27}H_{23}NO^{35}Cl$ requires *M*, 412.1468.

1-(4″-[{1‴-Methylimidazol-2‴-yl}methyl]piperazin-1″-yl)-2-(4′-chlorophenyl)-2-phenylethan-1-one 111

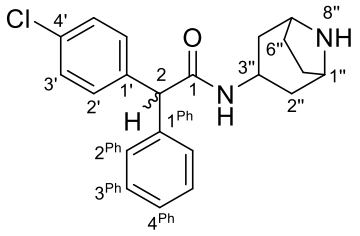


Procedure: Acid **90** (50 mg, 0.20 mmol) and 4-[{1′-methylimidazol-2′-yl}methyl]piperazine **113** (36 mg, 0.20 mmol) were reacted according to general procedure A. Following reversed-phase

column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (30 mg, 37%) was isolated as an orange-brown oil.

ν_{max} (ATR) 3061 (w), 3026 (w), 2942 (w), 2817 (w), 1640 (s), 1434 (m), 1366 (m), 1267 (m), 1199 (s), 1126 (s), 1032 (m) cm^{-1}; δ_H (600 MHz, $CDCl_3$) 7.34 – 7.30 (2H, m, 3^{Ph}-***H***$_2$), 7.28 – 7.24 (3H, m, 3′-***H***$_2$, 4^{Ph}-***H***), 7.21 – 7.17 (2H, m, 2^{Ph}-***H***$_2$), 7.14 – 7.10 (3H, m, 2′-***H***$_2$, 4‴-***H***), 6.94 (1H, d, *J* = 1.0 Hz, 5‴-***H***), 5.13 (1H, s, 2-***H***), 3.81 – 3.70 (3H, m, 2″-***H***H′, NC***H***$_2$), 3.77 (3H, s, NC***H***$_3$), 3.68 – 3.60 (1H, m, 2″-H***H***′), 3.43 – 3.34 (2H, m, 2″-***H***$_2$), 2.55 – 2.45 (2H, m, 3″-***H***$_2$(HH′)), 2.33 – 2.27 (1H, m, 3″-H_2(***H***H′)), 2.23 – 2.16 (1H, m, 3″-H_2(H***H***′)); δ_C (151 MHz, $CDCl_3$) 170.0 (***C***=O), 143.7 (***C***-2‴), 138.7 (***C***-1^{Ph}), 138.1 (***C***-1′), 133.1 (***C***-4′), 130.6 (***C***-2′), 129.0 (***C***-3^{Ph}), 128.8(2) (Ar***C***), 128.7(8) (Ar***C***), 127.5 (Ar***C***), 123.7 (***C***-4‴), 122.4 (***C***-5‴), 54.4 (***C***-2), 52.9 (***C***-3″), 52.6 (N***C***H_2), 52.5 (***C***-3″), 45.8 (***C***-2″), 42.1 (***C***-2″), 33.9 (N***C***H_3); *m/z* (LCMS, ESI^+) 409 ($M(^{35}Cl)H^+$), 411 ($M(^{37}Cl)H^+$); Accurate mass: Found MH^+, 409.1799: $C_{23}H_{26}N_4O^{35}Cl$ requires *M*, 409.1795.

***N*-(8″-Azabicyclo[3.2.1]octan-3″-yl)-2-(4′-chlorophenyl)-2-phenylacetamide 117**

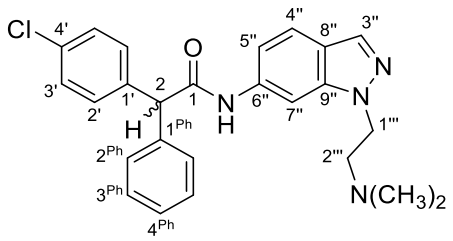


Procedure: Acid **90** (50 mg, 0.20 mmol) and 8-boc-8-azabicyclo[3.2.1]octanylamine (45 mg, 0.20 mmol) were reacted according to general procedure A. Following TFA-mediated deprotection and reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (12 mg, 17%) was isolated as a white solid.

M.p. 189 – 191 °C (decomp.); ν_{max} (ATR) 3278 (br), 3066 (w), 2958 (w), 2876 (w), 1644 (s), 1550 (m), 1490 (s), 1408 (w), 1312 (w), 1226 (w), 1162 (w), 1090 (m), 1015 (m) cm^{-1}; δ_H (600 MHz, $CDCl_3$) 7.33 – 7.28 (2H, m, 3^{Ph}***H***$_2$), 7.28 – 7.23 (3H, m, 3′-***H***$_2$, 4^{Ph}-***H***), 7.20 – 7.17 (2H, m, 2^{Ph}-***H***$_2$), 7.17 – 7.15 (2H, m, 2′-***H***$_2$), 5.36 (1H, d, *J* = 8.5 Hz, N***H***), 4.79 (1H, s, 2-***H***), 4.21 (1H, tdt, *J* = 11.5,

8.5, 5.5 Hz, 3''-***H***), 3.53 – 3.49 (2H, m, 1''-$\boldsymbol{H}_2$), 1.92 – 1.83 (2H, m, 2''-$\boldsymbol{H}_2$H'$_2$), 1.78 – 1.73 (4H, m, 6''-($\boldsymbol{H}_2$)$_2$), 1.72 – 1.61 (1H, m, 8''-***H***), 1.29 – 1.21 (2H, m, 2''-H$_2$***H***'$_2$); δ_C (151 MHz, $CDCl_3$) 170.7 (***C***=O), 139.1 (***C***-1^{Ph}), 138.1 (***C***-1'), 133.2 (***C***-4'), 130.3 (***C***-2'), 129.0 (***C***-3^{Ph}), 128.9 (***C***-3'), 128.8 (***C***-2^{Ph}), 127.6 (***C***-4^{Ph}), 58.5 (***C***-2), 54.6 (***C***-1''), 42.1 (***C***-3''), 39.8(3) (***C***-2''), 39.8(1) (***C***-2''), 29.5 (***C***-6''); *m/z* (LCMS, ESI$^+$) 355 (M(^{35}Cl)H$^+$), 357 (M(^{37}Cl)H$^+$); Accurate mass: Found MH$^+$, 355.1563: $C_{21}H_{24}N_2O^{35}Cl$ requires *M*, 355.1577.

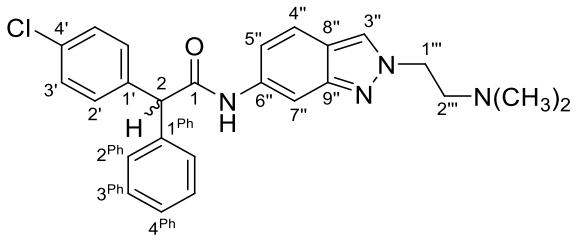
***N*-(1''-[2'''-Dimethylaminoethyl]indazol-6''-yl)-2-(4'-chlorophenyl)-2-phenylacetamide 118**



Procedure: Acid **90** (50 mg, 0.20 mmol) and 1-(2'-dimethylaminoethyl)-6-aminoindazole (41 mg, 0.20 mmol) were reacted according to general procedure A. Following reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (8 mg, 9%) was isolated as a yellow oil.

ν_{max} (ATR) 3306 (br), 3264 (w), 3070 (w), 2952 (w), 2862 (w), 2818 (w), 2780 (w), 1661 (m), 1625 (m), 1587 (m), 1546 (m), 1489 (s), 1462 (m), 1359 (m), 1310 (m), 1256 (w), 1167 (m), 1092 (m) cm^{-1}; δ_H (600 MHz, $CDCl_3$) 8.23 (1H, br s, 7''-***H***), 7.91 (1H, s, 3''-***H***), 7.57 (1H, d, *J* = 8.5 Hz, 4''-***H***), 7.45 (1H, br s, N***H***), 7.42 – 7.37 (2H, m, 3^{Ph}-$\boldsymbol{H}_2$), 7.36 – 7.31 (5H, m, 3'-$\boldsymbol{H}_2$, 2^{Ph}-$\boldsymbol{H}_2$, 4^{Ph}-***H***), 7.30 – 7.27 (2H, m, 2'-$\boldsymbol{H}_2$), 6.68 (1H, dd, *J* = 8.5, 1.5 Hz, 5''-***H***), 5.08 (1H, s, 2-***H***), 4.43 (2H, t, *J* = 7.0 Hz, 1'''-$\boldsymbol{H}_2$), 2.81 (2H, t, *J* = 7.0 Hz, 2'''-$\boldsymbol{H}_2$), 2.29 (6H, s, N(C$\boldsymbol{H}_3$)$_2$); δ_C (151 MHz, $CDCl_3$) 169.9 (***C***=O), 140.1 (***C***-9''), 138.6 (***C***-1^{Ph}), 137.5 (***C***-1'), 136.0 (***C***-6''), 133.7 (***C***-4'), 133.1 (***C***-3''), 130.5 (***C***-2'), 129.4 (***C***-3^{Ph}), 129.2 (***C***-3'), 129.0 (***C***-2^{Ph}), 128.1 (***C***-4^{Ph}), 121.7 (***C***-4''), 121.2 (***C***-8''), 114.2 (***C***-5''), 99.4 (***C***-7''), 59.7 (***C***-2), 58.5 (***C***-2'''), 47.2 (***C***-1'''), 45.9 (N(***C***H$_3$)); *m/z* (LCMS, ESI$^+$) 433 (M(^{35}Cl)H$^+$), 435 (M(^{37}Cl)H$^+$); Accurate mass: Found MH$^+$, 433.1769: $C_{25}H_{26}N_4O^{35}Cl$ requires *M*, 433.1795.

***N*-(2′′-[2′′′-Dimethylaminoethyl]indazol-6′′-yl)-2-(4′-chlorophenyl)-2-phenylacetamide 119**

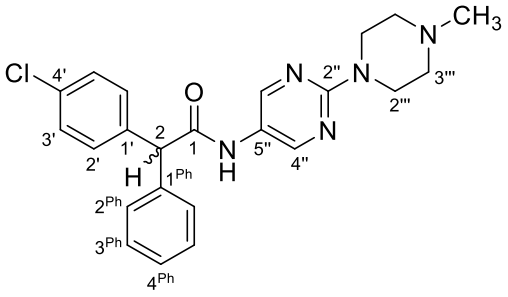


Procedure: Acid **90** (50 mg, 0.20 mmol) and 2-(2′-dimethylaminoethyl)-6-aminoindazole (41 mg, 0.20 mmol) were reacted according to general procedure A. Following reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (31 mg, 36%) was isolated as a light brown solid.

M.p. 76 – 79 °C; ν_{max} (ATR) 3292 (br), 3046 (w), 2950 (w), 2838 (w), 2790 (w), 1672 (m), 1569 (s), 1488 (s), 1368 (m), 1226 (m), 1148 (w), 1088 (w), 1014 (w) cm^{-1}; δ_H (600 MHz, $CDCl_3$) 7.94 (1H, s, 3′′-***H***), 7.93 (1H, br s, 7′′-***H***), 7.55 (1H, d, *J* = 9.0 Hz, 4′′-***H***), 7.40 – 7.36 (2H, m, 3^{Ph}-***H***$_2$), 7.35 – 7.31 (5H, m, 3′-***H***$_2$, 2^{Ph}-***H***$_2$, 4^{Ph}-***H***), 7.30 – 7.27 (2H, m, 2′-***H***$_2$), 7.02 (1H, dd, *J* = 9.0, 2.0 Hz, 5′′-***H***), 5.06 (1H, s, 2-***H***), 4.46 (2H, t, *J* = 6.5 Hz, 1′′′-***H***$_2$), 2.87 (2H, t, *J* = 6.5 Hz, 2′′′-***H***$_2$), 2.27 (6H, s, N(C***H***$_3$)$_2$); δ_C (151 MHz, $CDCl_3$) 169.6 (***C***=O), 148.9 (***C***-9′′), 138.9 (***C***-1^{Ph}), 137.8 (***C***-1′), 135.3 (***C***-6′′), 133.5 (***C***-4′), 130.5 (***C***-2′), 129.3 (***C***-3^{Ph}), 129.1 (***C***-3′), 129.0 (***C***-2^{Ph}), 127.9 (***C***-4^{Ph}), 123.5 (***C***-3′′), 121.0 (***C***-4′′), 119.5 (***C***-8′′), 117.1 (***C***-5′′), 106.7 (***C***-7′′), 59.6 (***C***-2), 59.4 (***C***-2′′′), 52.0 (***C***-1′′′), 45.8 (N(***C***H$_3$)$_2$); *m/z* (LCMS, ESI^+) 433 ($M(^{35}Cl)H^+$), 435 ($M(^{37}Cl)H^+$); Accurate mass: Found MH^+, 433.1783: $C_{25}H_{26}N_4O^{35}Cl$ requires *M*, 433.1795.

***N*-(2''-[4'''-Methylpiperazin-1'''-yl]pyrimidin-5''-yl)-2-(4'-chlorophenyl)-2-phenylacetamide**

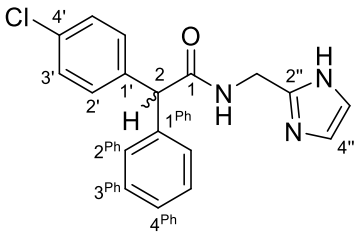
120



Procedure: Acid **90** (50 mg, 0.20 mmol) and 2-[4'-methylpiperazin-1'-yl]-5-aminopyrimidine (39 mg, 0.20 mmol) were reacted according to general procedure A. Following reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (30 mg, 36%) was isolated as an off-white solid.

M.p. 152 – 154 °C; ν_{max} (ATR) 3270 (br), 2942 (w), 2846 (w), 2800 (w), 1655 (m), 1606 (m), 1498 (br, s), 1447 (m), 1407 (m), 1360 (s), 1300 (m), 1261 (m), 1170 (w), 1096 (w) cm^{-1}; δ_H (600 MHz, $CDCl_3$) 8.37 (2H, s, 4''-***H***$_2$), 7.39 – 7.35 (2H, m, 3^{Ph}-***H***$_2$), 7.34 – 7.31 (3H, m, 3'-***H***$_2$, 4^{Ph}-***H***), 7.31 – 7.28 (2H, m, 2^{Ph}-***H***$_2$), 7.26 – 7.23 (2H, m, 2'-***H***$_2$), 7.01 (1H, s, N***H***), 5.01 (1H, s, 2-***H***), 3.80 (4H, t, *J* = 5.0 Hz, 2'''-(***H***$_2$)$_2$), 2.43 (4H, t, *J* = 5.0 Hz, 3'''-(***H***$_2$)$_2$), 2.32 (3H, s, NC***H***$_3$); δ_C (151 MHz, $CDCl_3$) 170.2 (***C***=O), 159.7 (***C***-2''), 151.6 (***C***-4''), 138.6 (***C***-1^{Ph}), 137.5 (***C***-1'), 133.7 (***C***-4'), 130.4 (***C***-2'), 129.3 (***C***-3^{Ph}), 129.2 (***C***-3'), 128.9 (***C***-2^{Ph}), 128.0 (***C***-4^{Ph}), 122.3 (***C***-5''), 58.8 (***C***-2), 55.0 (***C***-3'''), 46.4 (N***C***H_3), 44.2 (***C***-2'''); *m/z* (LCMS, ESI^+) 422 ($M(^{35}Cl)H^+$), 424 ($M(^{37}Cl)H^+$); Accurate mass: Found MH^+, 422.1757: $C_{23}H_{25}N_5O^{35}Cl$ requires *M*, 422.1748.

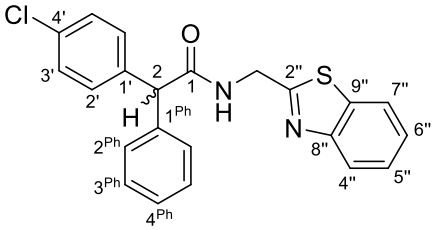
***N*-([1″*H*-Imidazol-2″-yl]methyl)-2-(4′-chlorophenyl)-2-phenylacetamide 121**



Procedure: Acid **90** (50 mg, 0.20 mmol) and (1*H*-imidazol-2-yl)methylamine·2HCl (34 mg, 0.20 mmol) were reacted according to general procedure A. Following reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (16 mg, 25%) was isolated as a beige solid.

M.p. 127 – 130 °C; ν_{max} (ATR) 3232 (br), 3032 (w), 2930 (w), 1656 (s), 1558 (w), 1490 (s), 1420 (w), 1133 (s), 1091 (s), 1015 (m) cm^{-1}; δ_H (600 MHz, $CDCl_3$) 9.14 – 9.08 (1H, m, N***H***), 7.26 – 7.23 (3H, m, 3^{Ph}-***H***$_2$, 4^{Ph}-***H***), 7.22 – 7.19 (2H, m, 3′-***H***$_2$), 7.11 – 7.07 (2H, m, 2^{Ph}-***H***$_2$), 7.07 – 7.04 (2H, m, 2′-***H***$_2$), 6.89 (2H, s, 4′′-(***H***)$_2$,), 4.83 (1H, s, 2-***H***), 4.46 (2H, d, *J* = 6.0 Hz, NHC***H***$_2$); δ_C (151 MHz, $CDCl_3$) 174.3 (***C***=O), 145.2 (***C***-2′′), 138.6 (***C***-1^{Ph}), 137.4 (***C***-1′), 133.4 (***C***-4′), 130.2 (***C***-2′), 128.9(1) (***C***-3^{Ph}), 128.8(8) (***C***-3′), 128.7 (***C***-2^{Ph}), 127.7 (***C***-4^{Ph}), 120.4 (***C***-4′′), 57.2 (***C***-2), 35.9 (NH***C***H_2); *m/z* (LCMS, ESI^+) 326 ($M(^{35}Cl)H^+$), 328 ($M(^{37}Cl)H^+$); Accurate mass: Found MH^+, 326.1050: $C_{18}H_{17}N_3O^{35}Cl$ requires *M*, 326.1060.

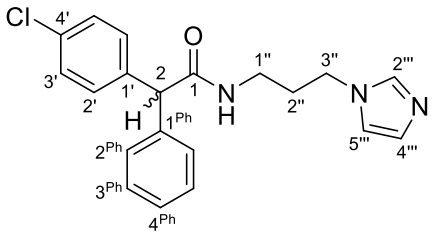
***N*-([Benzothiazol-2″-yl]methyl)-2-(4′-chlorophenyl)-2-phenylacetamide 122**



Procedure: Acid **90** (50 mg, 0.20 mmol) and (benzothiazol-2-yl)methylamine·HCl (40 mg, 0.20 mmol) were reacted according to general procedure A. Following reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (18 mg, 23%) was isolated as a beige solid.

M.p. 161 – 163 °C; ν_{max} (ATR) 3294 (br), 3062 (w), 3018 (w), 1654 (s), 1528 (m), 1489 (s), 1412 (w), 1334 (w), 1218 (w), 1156 (w), 1090 (m), 1015 (m) cm^{-1}; δ_H (600 MHz, $CHCl_3$) 7.94 (1H, d, *J* = 8.0 Hz, 7''-***H***), 7.85 (1H, d, *J* = 8.0 Hz, 4''-***H***), 7.47 (1H, t, *J* = 8.0 Hz, 5''-***H***), 7.39 (1H, t, *J* = 8.0 Hz, 6''-***H***), 7.37 – 7.30 (2H, m, 3^{Ph}-$\boldsymbol{H}_2$), 7.33 – 7.26 (5H, m, 3'-$\boldsymbol{H}_2$, 2^{Ph}-$\boldsymbol{H}_2$, 4^{Ph}-***H***), 7.28 – 7.22 (2H, m, 2'-$\boldsymbol{H}_2$), 6.59 (1H, t, *J* = 6.0 Hz, N***H***), 4.98 (1H, s, 2-***H***), 4.89 – 4.85 (2H, m, NHC$\boldsymbol{H}_2$); δ_C (151 MHz, $CDCl_3$) 171.8 (***C***=O), 167.9 (***C***-2''), 152.8 (***C***-8''), 138.7 (***C***-1^{Ph}), 137.7 (***C***-1'), 135.3 (***C***-9''), 133.5 (***C***-4'), 130.5 (***C***-2'), 129.2 (***C***-3^{Ph}), 129.1 (Ar***C***), 129.0 (Ar***C***), 127.8 (***C***-4^{Ph}), 126.4 (***C***-5''), 125.5 (***C***-6''), 123.0 (***C***-7''), 121.9 (***C***-4''), 58.4 (***C***-2), 42.3 (NH***C***H_2); *m/z* (LCMS, ESI^+) 393 ($M(^{35}Cl)H^+$), 395 ($M(^{37}Cl)H^+$); Accurate mass: Found MH^+, 393.0827: $C_{22}H_{18}N_2OS^{35}Cl$ requires *M*, 393.0828.

***N*-(3''-[Imidazol-1'''-yl]prop-1''-yl)-2-(4'-chlorophenyl)-2-phenylacetamide 123**

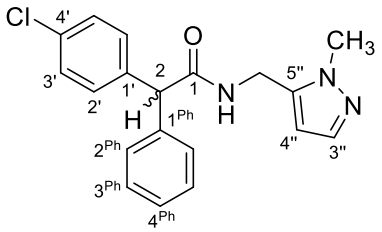


Procedure: Acid **90** (50 mg, 0.20 mmol) and 3-[imidazol-1'-yl]propylamine (98%, 26 mg, 0.20 mmol) were reacted according to general procedure A. Following reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (30 mg, 42%) was isolated as a white solid.

M.p. 116 – 118 °C; ν_{max} (ATR) 3312 (br), 3044 (w), 2952 (w), 1650 (s), 1560 (m), 1489 (s), 1458 (w), 1352 (w), 1226 (m), 1098 (w) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 7.58 (1H, s, 2'''-***H***), 7.32 (2H, t, *J* = 7.5 Hz, 3^{Ph}-$\boldsymbol{H}_2$), 7.28 (2H, d, *J* = 8.0 Hz, 3'-$\boldsymbol{H}_2$), 7.26 – 7.23 (3H, m, 2^{Ph}-$\boldsymbol{H}_2$, 4^{Ph}-***H***), 7.20 (2H, d, *J* = 8.0 Hz, 2'-$\boldsymbol{H}_2$), 7.02 (1H, apparent s, 4'''-***H***), 6.89 (1H, apparent s, 5'''-***H***), 6.31 (1H, t, *J* = 6.5 Hz, N***H***), 4.86 (1H, s, 2-***H***), 3.93 (2H, t, *J* = 7.0 Hz, 3''-$\boldsymbol{H}_2$), 3.25 (2H, q, *J* = 6.5 Hz, 1''-$\boldsymbol{H}_2$), 1.97 (2H, tt, *J* = 7.0, 6.5 Hz, 2''-$\boldsymbol{H}_2$); δ_C (176 MHz, $CDCl_3$) 172.2 (***C***=O), 139.1 (***C***-1^{Ph}), 137.9 (***C***-1'), 137.1 (***C***-2'''), 133.3 (***C***-4'), 130.3 (***C***-2'), 129.1 (***C***-3^{Ph}), 129.0 (***C***-3'), 128.8 (***C***-2^{Ph}), 128.7 (***C***-4'''),

119.2 (***C***-5‴), 58.2 (***C***-2), 44.9 (***C***-3″), 37.0 (***C***-1″), 31.0 (***C***-2″); *m/z* (LCMS, ESI^+) 354 ($M(^{35}Cl)H^+$), 356 ($M(^{37}Cl)H^+$); Accurate mass: Found MH^+, 354.1383: $C_{20}H_{21}N_3O^{35}Cl$ requires *M*, 354.1373.

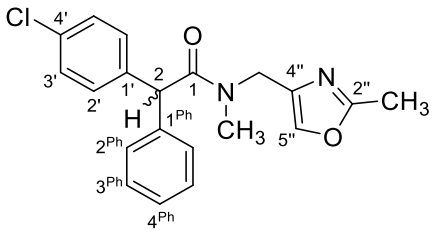
_N_-([_N_-Methylpyrazol-5″-yl]methyl)-2-(4′-chlorophenyl)-2-phenylacetamide 124



Procedure: Acid **90** (50 mg, 0.20 mmol) and (*N*-methylpyrazol-5-yl)methylamine (97%, 23 mg, 0.20 mmol) were reacted according to general procedure A. Following reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (25 mg, 37%) was isolated as a white solid.

M.p. 148 – 150 °C; ν_{max} (ATR) 3274 (br), 3030 (w), 2934 (w), 1648 (s), 1544 (m), 1490 (s), 1400 (w), 1344 (w), 1280 (w), 1218 (w), 1090 (m), 1015 (m) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 7.37 (1H, d, *J* = 2.0 Hz, 3″-***H***), 7.36 – 7.33 (2H, m, 3^{Ph}-$\boldsymbol{H}_2$), 7.31 – 7.27 (3H, m, 3′-$\boldsymbol{H}_2$, 4^{Ph}-***H***), 7.24 – 7.22 (2H, m, 2^{Ph}-$\boldsymbol{H}_2$), 7.21 – 7.18 (2H, m, 2′-$\boldsymbol{H}_2$), 6.05 (1H, d, *J* = 2.0 Hz, 4″-***H***), 5.79 (1H, t, *J* = 5.5 Hz, N-***H***), 4.87 (1H, s, 2-***H***), 4.52 (2H, d, *J* = 5.5 Hz, NHC$\boldsymbol{H}_2$), 3.77 (3H, s, NC$\boldsymbol{H}_3$); δ_C (101 MHz, $CDCl_3$) 171.4 (***C***=O), 138.6(3) (Ar***C***), 138.5(9) (Ar***C***), 138.4 (***C***-3″), 137.6 (***C***-1′), 133.5 (***C***-4′), 130.3 (***C***-2′), 129.1 (Ar***C***), 129.0 (Ar***C***), 128.7 (***C***-2^{Ph}), 127.9 (***C***-4^{Ph}), 105.8 (***C***-4″), 58.3 (***C***-2), 36.6 (N***C***H_3), 34.6 (NH***C***H_2); *m/z* (LCMS, ESI^+) 340 ($M(^{35}Cl)H^+$), 342 ($M(^{37}Cl)H^+$); Accurate mass: Found MH^+, 340.1226: $C_{19}H_{19}N_3O^{35}Cl$ requires *M*, 340.1217.

_N_-Methyl-_N_-([2''-methyloxazol-4''-yl]methyl)-2-(4'-chlorophenyl)-2-phenylacetamide 125

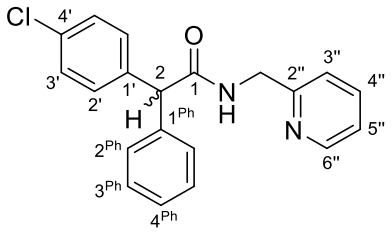


Procedure: Acid **90** (50 mg, 0.20 mmol) and *N*-methyl-(2-methyloxazol-4-yl)methylamine (25 mg, 0.20 mmol) were reacted according to general procedure A. Following reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (35 mg, 49%) was isolated as a clear yellow oil.

ν_{max} (ATR) 3050 (w), 2928 (w), 1643 (s), 1580 (m), 1489 (s), 1397 (m), 1278 (w), 1204 (w), 1089 (s), 1015 (m) cm^{-1}; δ_H (600 MHz, $CDCl_3$, mixture of rotamers) 7.42 (0.6H, s, 5''-***H*** rotamer B), 7.32 – 7.28 (2H, m, 3^{Ph}-***H***$_2$), 7.27 – 7.23 (4.2H, m, 3'-***H***$_2$, 2^{Ph}-***H***$_2$ rotamer A, 4^{Ph}-***H***, 5''-***H*** rotamer A), 7.21 – 7.18 (2H, m, 2'-***H***$_2$ rotamer A, 2^{Ph}-***H***$_2$ rotamer B), 7.15 – 7.12 (1.2H, m, 2'-***H***$_2$, rotamer B), 5.49 (0.4H, s, 2-***H*** rotamer A), 5.15 (0.6H, s, 2-***H*** rotamer B), 4.45 (1.2H, s, $N(CH_3)C\boldsymbol{H}_2$ rotamer B), 4.36 (0.4H, d, *J* = 17.0 Hz, $N(CH_3)C\boldsymbol{H}$H' rotamer A), 4.21 (0.4H, d, *J* = 17.0 Hz, $N(CH_3)H\boldsymbol{H}$' rotamer A), 3.05 (1.8H, s, $N(C\boldsymbol{H}_3)CH_2$ rotamer B), 3.00 (1.2H, s, $N(C\boldsymbol{H}_3)$CHH' rotamer A), 2.46 (1.2H, s, 2''-$C\boldsymbol{H}_3$ rotamer A), 2.41 (1.8H, s, 2''-$C\boldsymbol{H}_3$ rotamer B); δ_C (151 MHz, $CDCl_3$, mixture of rotamers) 171.8 (***C***=O rotamer A), 171.4 (***C***=O rotamer B), 162.5 (***C***-2'' rotamer A), 161.5 (***C***-2'' rotamer B), 139.1 (***C***-1^{Ph} rotamer A), 138.9 (***C***-1^{Ph} rotamer B), 138.5 (***C***-1' rotamer A), 138.2 (***C***-1' rotamer B), 137.0 (***C***-4'' rotamer A), 136.8 (***C***-4'' rotamer B), 136.2 (***C***-5'' rotamer B), 135.1 (***C***-5'' rotamer A), 133.0(4) (***C***-4' rotamer B), 133.0(1) (***C***-4' rotamer A), 130.7 (***C***-2' rotamer A), 130.6 (***C***-2' rotamer B), 129.0 (Ar***C***), 128.9(1) (Ar***C***), 128.8(8) (Ar***C***), 128.8(6) (Ar***C***), 128.7(1) (Ar***C***), 128.6(5) (Ar***C***), 127.4(1) (***C***-4^{Ph} rotamer A), 127.3(8) (***C***-4^{Ph} rotamer B), 54.3 (***C***-2 rotamer B), 53.8 (***C***-2 rotamer A), 46.1 ($N(CH_3)\boldsymbol{C}$HH' rotamer A), 44.0 ($N(CH_3)\boldsymbol{C}H_2$ rotamer B), 36.2 ($N(\boldsymbol{C}H_3)CH_2$ rotamer B), 34.3 ($N(\boldsymbol{C}H_3)$CHH' rotamer A), 14.1 (2''-$\boldsymbol{C}H_3$ rotamer A), 14.0

(2′′-***C***H_3 rotamer B); *m/z* (LCMS, ESI^+) 355 ($M(^{35}Cl)H^+$), 357 ($M(^{37}Cl)H^+$); Accurate mass: Found MH^+, 355.1220: $C_{20}H_{20}N_2O_2{}^{35}Cl$ requires *M*, 355.1213.

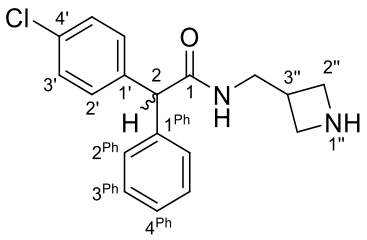
_N_-([Pyridin-2′′-yl]methyl)-2-(4′-chlorophenyl)-2-phenylacetamide 126



Procedure: Acid **90** (50 mg, 0.20 mmol) and (pyridin-2-yl)methylamine (22 mg, 0.20 mmol) were reacted according to general procedure A. Following reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (47 mg, 70%) was isolated as a white solid.

M.p. 119 – 120 °C; ν_{max} (ATR) 3308 (br), 3056 (w), 2930 (w), 1649 (s), 1600 (w), 1591 (m), 1490 (s), 1437 (w), 1204 (w), 1091 (m), 1016 (m) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 8.48 – 8.46 (1H, m, 6′′-***H***), 7.64 (1H, td, *J* = 7.5, 2.0 Hz, 5′′-***H***), 7.35 – 7.31 (2H, m, 3^{Ph}-***H***$_2$), 7.30 – 7.26 (5H, m, 3′-***H***$_2$, 2^{Ph}-***H***$_2$, 4^{Ph}-***H***), 7.26 – 7.24 (2H, m, 2′-***H***$_2$), 7.23 – 7.21 (1H, m, 3′′-***H***), 7.20 – 7.16 (1H, m, 4′′-***H***), 6.97 – 6.92 (1H, m, N-***H***), 4.96 (1H, s, 2-***H***), 4.59 (2H, d, *J* = 5.0 Hz, NHC***H***$_2$); δ_C (176 MHz, $CDCl_3$) 171.6 (***C***=O), 156.1 (***C***-2′′), 149.1 (***C***-6′′), 139.2 (***C***-1^{Ph}), 138.2 (***C***-1′), 136.9 (***C***-5′′), 133.2 (***C***-4′), 130.4 (***C***-2′), 129.0 (***C***-3^{Ph}), 128.9(3) (Ar***C***), 128.9(1) (Ar***C***), 127.6 (***C***-4^{Ph}), 122.6 (***C***-4′′), 122.2 (***C***-3′′), 58.5 (***C***-2), 44.8 (NH***C***H_2); *m/z* (LCMS, ESI^+) 337 ($M(^{35}Cl)H^+$), 339 ($M(^{37}Cl)H^+$); Accurate mass: Found MH^+, 337.1111: $C_{20}H_{18}N_2O^{35}Cl$ requires *M*, 337.1108.

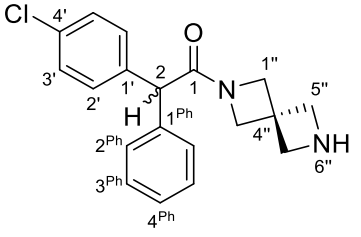
_N_-([Azetidin-3''-yl]methyl)-2-(4'-chlorophenyl)-2-phenylacetamide 127



Procedure: Acid **90** (50 mg, 0.20 mmol) and (*N*-boc-azetidin-3-yl)methylamine (37 mg, 0.20 mmol) were reacted according to general procedure A. Following TFA-mediated deprotection and reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (10 mg, 16%) was isolated as a yellow solid.

M.p. 102 – 105 °C; ν_{max} (ATR) 3284 (br), 3066 (w), 2956 (w), 2924 (w), 2866 (w), 1649 (s), 1555 (m), 1490 (s), 1364 (w), 1226 (w), 1098 (w), 1018 (w) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 7.35 – 7.30 (2H, m, 3^{Ph}-***H***$_2$), 7.30 – 7.26 (3H, m, 3'-***H***$_2$, 4^{Ph}-***H***), 7.25 – 7.22 (2H, m, 2^{Ph}-***H***$_2$), 7.22 – 7.19 (2H, m, 2'-***H***$_2$), 6.21 – 6.13 (1H, m, N***H***), 4.88 (1H, s, 2-***H***), 3.72 – 3.63 (2H, m, 2''-***H***$_2$H'$_2$), 3.47 (2H, t, *J* = 6.0 Hz, NHC***H***$_2$), 3.29 – 3.20 (2H, m, 2''-H$_2$***H***'$_2$), 2.86 – 2.78 (1H, m, 3''-***H***), 2.04 – 1.87 (1H, m, 1''-***H***); δ_C (176 MHz, $CDCl_3$) 172.0 (***C***=O), 139.2 (***C***-1^{Ph}), 138.1 (***C***-1'), 133.3 (***C***-4'), 130.4 (***C***-2'), 129.0 (***C***-3^{Ph}), 128.9 (***C***-3'), 128.8 (***C***-2^{Ph}), 127.6 (***C***-4^{Ph}), 58.6 (***C***-2), 50.9 (***C***-2''), 43.0 (NH***C***H$_2$), 34.5 (***C***-3''); *m/z* (LCMS, ESI^+) 315 (M(^{35}Cl)H^+), 317 (M(^{37}Cl)H^+); Accurate mass: Found MH^+, 315.1257: $C_{18}H_{20}N_2O^{35}Cl$ requires *M*, 315.1264.

1-(2'',6''-Diazaspiro[3.3]heptan-2''-yl)-2-(4'-chlorophenyl)-2-phenylethan-1-one 128

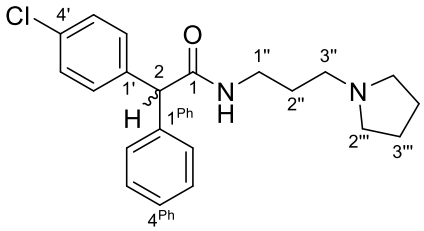


Procedure: Acid **90** (50 mg, 0.20 mmol) and 2-boc-2,6-diazaspiro[3.3]heptane (40 mg, 0.20 mmol) were reacted according to general procedure A. Following reversed-phase column

chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (25 mg, 38%) was isolated as a white crystalline solid.

M.p. 90 – 92 °C; ν_{max} (ATR) 2942 (w), 2866 (w), 1645 (s), 1490 (m), 1437 (s), 1326 (w), 1290 (w), 1152 (w), 1092 (w), 1015 (w) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 7.33 – 7.29 (2H, m, 3^{Ph}-***H***$_2$), 7.28 – 7.25 (5H, m, 3′-***H***$_2$, 2^{Ph}-***H***$_2$, 4^{Ph}-***H***), 7.24 – 7.21 (2H, m, 2′-***H***$_2$), 4.77 (1H, s, 2-***H***), 4.22 (1H, d, *J* = 9.0 Hz, 1′′-(***H***H′)(H$_2$)), 4.15 (1H, d, *J* = 9.0 Hz, 1′′-(H***H***′)(H$_2$)), 4.11 (2H, s, 1′′-(HH′)***H***$_2$), 3.77 (1H, d, *J* = 8.5 Hz, 5′′-(***H***H′)(HH′)), 3.75 (1H, d, *J* = 8.5 Hz, 5′′-(HH′)(***H***H′)), 3.68 (1H, d, *J* = 8.5 Hz, 5′′-(H***H***′)(HH′)), 3.65 (1H, d, *J* = 8.5 Hz, 5′′-(HH′)(H***H***′)); δ_C (176 MHz, $CDCl_3$) 171.0 (***C***=O), 138.6 (***C***-1^{Ph}), 137.6 (***C***-1′), 133.2 (***C***-4′), 130.4 (***C***-2′), 128.9 (***C***-3^{Ph}), 128.7(9) (Ar***C***), 128.7(7) (Ar***C***), 127.5 (***C***-4^{Ph}), 61.5 (***C***-1′′), 58.6 (***C***-1′′), 57.6(4) (Al***C***), 57.5(5) (Al***C***), 52.9 (***C***-2), 37.4 (***C***-4′′); *m/z* (LCMS, ESI^+) 327 ($M(^{35}Cl)H^+$), 329 ($M(^{37}Cl)H^+$); Accurate mass: Found MH^+, 327.1248: $C_{19}H_{20}N_2O^{35}Cl$ requires *M*, 327.1264.

_N_-(3′′-[Pyrrolidin-1′′′-yl]prop-1′′-yl)-2-(4′-chlorophenyl)-2-phenylacetamide 129

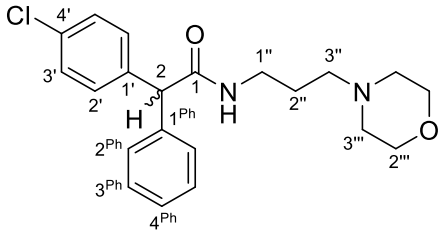


Procedure: Acid **90** (50 mg, 0.20 mmol) and 3-(pyrrolidin-1′-yl)propylamine (26 mg, 0.20 mmol) were reacted according to general procedure A. Following reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (29 mg, 41%) was isolated as a yellow oil.

ν_{max} (ATR) 3278 (br), 3064 (w), 2968 (w), 2930 (w), 2880 (w), 2788 (w), 1654 (s), 1548 (m), 1494 (s), 1452 (w), 1352 (w), 1222 (w), 1176 (w), 1096 (w), 1015 (w) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 7.45 – 7.41 (1H, m, N***H***), 7.34 – 7.30 (2H, m, Ar***H***), 7.29 – 7.26 (4H, m, Ar***H***), 7.26 – 7.23 (3H, m, Ar***H***), 4.81 (1H, s, 2-***H***), 3.42 – 3.38 (2H, m, 1′′-***H***$_2$), 2.61 (2H, t, *J* = 6.5 Hz, 3′′-***H***$_2$), 2.60 – 2.54 (4H, m,

2‴-(***H***$_2$)$_2$), 1.79 – 1.72 (6H, m, 2″-***H***$_2$, 3‴-(***H***$_2$)$_2$); δ_C (176 MHz, $CDCl_3$) 171.8 (***C***=O), 139.5 (Ar***C***), 138.4 (Ar***C***), 133.1 (***C***-4′), 130.4 (Ar***C***), 128.9 (Ar***C***), 128.8(4) (Ar***C***), 128.8(1) (Ar***C***), 127.4 (Ar***C***), 58.6 (***C***-2), 54.8 (***C***-3″), 54.1 (***C***-2‴), 39.3 (***C***-1″), 26.6 (***C***-2″), 23.5 (***C***-3‴); *m/z* (LCMS, ESI$^+$) 357 (M(^{35}Cl)H$^+$), 358 (M(^{37}Cl)H$^+$); Accurate mass: Found MH$^+$, 357.1741: $C_{21}H_{26}N_2O^{35}Cl$ requires *M*, 357.1734.

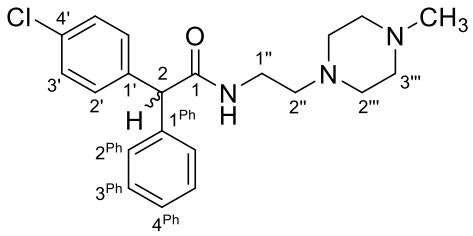
***N*-(3″-[Morpholin-4‴-yl]prop-1″-yl)-2-(4′-chlorophenyl)-2-phenylacetamide 130**



Procedure: Acid **90** (50 mg, 0.20 mmol) and 3-(morpholin-4′-yl)propylamine (29 mg, 0.20 mmol) were reacted according to general procedure A. Following reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (35 mg, 47%) was isolated as a white solid.

M.p. 129 – 131 °C; ν_{max} (ATR) 3290 (br), 3054 (w), 2942 (w), 2852 (w), 2808 (w), 1645 (s), 1544 (m), 1489 (s), 1456 (w), 1356 (w), 1276 (w), 1222 (w), 1117 (s), 1090 (w), 1015 (m) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 7.34 – 7.30 (2H, m, 3^{Ph}-***H***$_2$), 7.29 – 7.26 (3H, m, 3′-***H***$_2$, 4^{Ph}-***H***), 7.26 – 7.24 (2H, m, 2^{Ph}-***H***$_2$), 7.22 – 7.19 (2H, m, 2′-***H***$_2$), 6.82 (1H, t, *J* = 5.0 Hz, N***H***), 4.80 (1H, s, 2-***H***), 3.60 – 3.53 (4H, m, 2‴-(***H***$_2$)$_2$), 3.43 – 3.33 (2H, m, 1″-***H***$_2$), 2.36 (2H, t, *J* = 6.5 Hz, 3″-***H***$_2$), 2.38 – 2.29 (4H, m, 3‴-(***H***$_2$)$_2$), 1.65 (2H, p, *J* = 6.5 Hz, 2″-***H***$_2$); δ_C (176 MHz, $CDCl_3$) 171.3 (***C***=O), 139.4 (***C***-1^{Ph}), 138.3 (***C***-1′), 133.2 (***C***-4′), 130.3 (***C***-2′), 129.0 (***C***-3^{Ph}), 128.9 (***C***-3′), 128.8 (***C***-2^{Ph}), 127.6 (***C***-4^{Ph}), 67.1 (***C***-2‴), 58.7 (***C***-2), 57.6 (***C***-3″), 53.8 (***C***-3‴), 39.6 (***C***-1″), 25.0 (***C***-2″); *m/z* (LCMS, ESI$^+$) 373 (M(^{35}Cl)H$^+$), 375 (M(^{37}Cl)H$^+$); Accurate mass: Found MH$^+$, 373.1675: $C_{21}H_{26}N_2O_2{}^{35}Cl$ requires *M*, 373.1683.

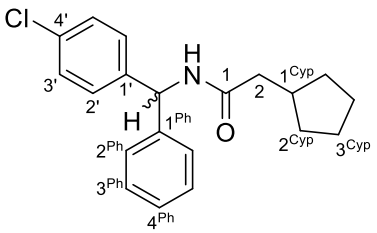
***N*-(2''-[4'''-Methylpiperazin-1'''-yl]eth-1''-yl)-2-(4'-chlorophenyl)-2-phenylacetamide 131**



Procedure: Acid **90** (50 mg, 0.20 mmol) and 2-(4'-methylpiperazin-1'-yl)ethylamine (29 mg, 0.20 mmol) were reacted according to general procedure A. Following reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (29 mg, 39%) was isolated as a pale yellow oil.

ν_{max} (ATR) 3308 (br), 2934 (w), 2800 (w), 1651 (s), 1544 (w), 1491 (s), 1456 (w), 1288 (w), 1176 (w), 1096 (w), 1015 (m) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 7.36 – 7.32 (2H, m, 3^{Ph}-***H***$_2$), 7.31 – 7.26 (3H, m, 3'-***H***$_2$, 4^{Ph}-***H***), 7.25 – 7.23 (2H, m, 2^{Ph}-***H***$_2$), 7.22 – 7.20 (2H, m, 2'-***H***$_2$), 6.28 – 6.18 (1H, m, N***H***), 4.90 (1H, s, 2-***H***), 3.40 – 3.31 (2H, m, 1''-***H***$_2$), 2.55 – 2.11 (8H, m, 2'''-(***H***$_2$)$_2$, 3'''-(***H***$_2$)$_2$), 2.44 (2H, t, *J* = 6.0 Hz, 2''-***H***$_2$), 2.27 (3H, s, NC***H***$_3$); δ_C (176 MHz, $CDCl_3$) 171.5 (***C***=O), 139.4 (***C***-1^{Ph}), 138.3 (***C***-1'), 133.2 (***C***-4'), 130.5 (***C***-2'), 129.0(3) (Ar***C***), 128.9(90) (Ar***C***), 128.9(85) (Ar***C***), 127.6 (***C***-4^{Ph}), 58.8 (***C***-2), 56.0 (***C***-2''), 55.1 (***C***-3'''), 52.6 (***C***-2'''), 46.1 (N***C***H_3), 36.3 (***C***-1''); *m/z* (LCMS, ESI^+) 372 ($M(^{35}Cl)H^+$), 374 ($M(^{37}Cl)H^+$); Accurate mass: Found MH^+, 372.1836: $C_{21}H_{27}N_3O^{35}Cl$ requires *M*, 372.1843.

***N*-([4'-Chlorophenyl]phenylmethyl)-2-cyclopentylacetamide 132**

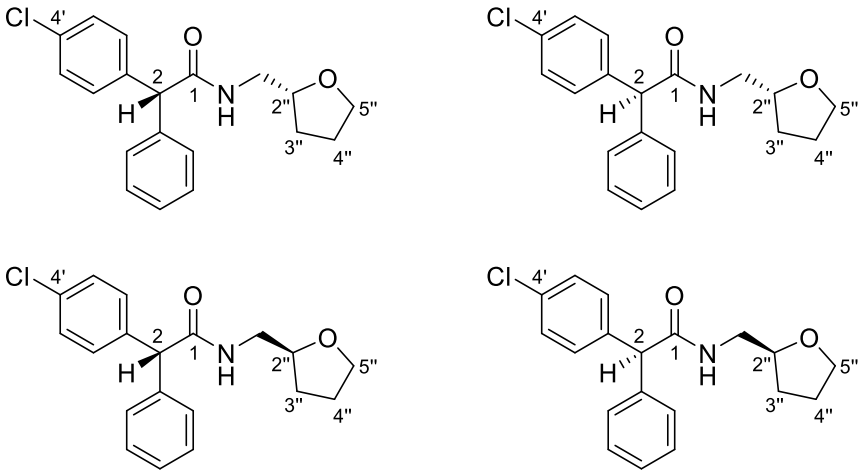


Procedure: Cyclopentylacetic acid (97%, 0.13 mL, 1 mmol) and 4-chlorobenzhydryl amine HCl salt **89** (98%, 285 mg, 1.1 mmol) were reacted according to general procedure A. Following SiO_2 column chromatography (0% → 50% EtOAc in hexanes), the *title compound* (260 mg, 79%) was isolated as clear needles.

R_f 0.36 (10% EtOAc in hexanes); M.p 146 – 148 °C; ν_{max} (ATR) 3274 (br), 3030 (w), 2949 (m), 2867 (w), 1637 (s), 1533 (m), 1490 (m), 1366 (m), 1217 (m), 1091 (m) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 7.33 (2H, t, *J* = 7.5 Hz, 3^{Ph}-***H***$_2$), 7.31 – 7.27 (3H, m, 3′-***H***$_2$, 4^{Ph}-***H***), 7.19 (2H, d, *J* = 7.5 Hz, 2^{Ph}-***H***$_2$), 7.16 (2H, d, *J* = 8.0 Hz, 2′-***H***$_2$), 6.23 (1H, d, *J* = 8.0 Hz, NHC***H***), 5.97 (1H, d, *J* = 8.0 Hz, N***H***CH), 2.29 – 2.23 (3H, m, 2-***H***$_2$, 1^{Cyp}-***H***), 1.86 – 1.80 (2H, m, 2^{Cyp}-***H***$_2$H′$_2$), 1.66 – 1.59 (2H, m, 3^{Cyp}-***H***$_2$H′$_2$), 1.59 – 1.52 (2H, m, 3^{Cyp}-H$_2$***H***′$_2$), 1.19 – 1.12 (2H, m, 2^{Cyp}-H$_2$***H***′$_2$); δ_C (176 MHz, $CDCl_3$) 172.0 (***C***-1), 141.3 (***C***-1^{Ph}), 140.3 (***C***-1′), 133.4 (***C***-4′), 129.0 (Ar***C***), 128.9 (Ar***C***), 128.8 (Ar***C***), 127.9 (***C***-4^{Ph}), 127.6 (***C***-2^{Ph}), 56.5 (NH***C***H), 43.2 (***C***-2), 37.3 (***C***-1^{Cyp}), 32.7(7) (***C***-2^{Cyp}), 32.7(6) (***C***-2^{Cyp}), 25.2 (***C***-3^{Cyp}); *m/z* (LCMS, ESI^+) 328 ($M(^{35}Cl)H^+$), 330 ($M(^{37}Cl)H^+$); Accurate mass: Found MH^+, 328.1484: $C_{20}H_{23}NO^{35}Cl$ requires *M*, 328.1468.

***N*-([Tetrahydrofuran-2″-yl]methyl)-2-(4′-chlorophenyl)-2-phenylacetamide 133**

A mixture of (2″*R*,2*R*), (2″*R*,2*S*), (2″*S*,2*R*) and (2″*S*,2*S*) diastereomers

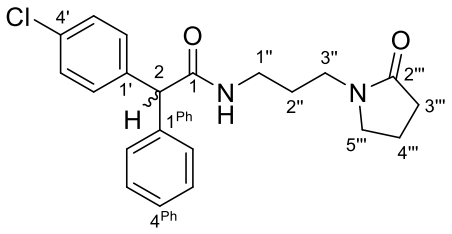


Procedure: Acid **90** (50 mg, 0.20 mmol) and (tetrahydrofuran-2-yl)methylamine (20 mg, 0.20 mmol) were reacted according to general procedure A. Following reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (44 mg, 66%) was isolated as an off-white solid.

M.p. 62 – 64 °C; ν_{max} (ATR) 3312 (br), 3086 (w), 3026 (w), 3026 (w), 2964 (w), 2875 (w), 1650 (s), 1544 (m), 1490 (s), 1448 (w), 1360 (w), 1222 (w), 1086 (m), 1015 (m) cm^{-1}; δ_H (400 MHz,

$CDCl_3$, mixture of diastereomers) 7.36 – 7.19 (9H, m, Ar***H***), 6.05 – 5.98 (1H, m, N***H***), 4.87 (0.5H, s, 2-***H***), 4.86 (0.5H, s, 2-***H***), 3.94 (1H, qd, *J* = 7.0, 3.5 Hz, 2''-***H***), 3.74 – 3.64 (2H, m, 5''-$\boldsymbol{H}_2$), 3.60 (0.5H, ddd, *J* = 5.5, 3.5, 2.5 Hz, NHC***H***H' isomer A), 3.56 (0.5H, ddd, *J* = 5.5, 3.5, 2.5 Hz, NHC***H***H' isomer B), 3.24 (0.5H, ddd, *J* = 7.0, 5.5, 4.5 Hz, NHCH***H***' isomer A), 3.21 (0.5H, ddd, *J* = 7.0, 5.5, 4.5 Hz, NHCH***H***' isomer B), 1.98 – 1.85 (1H, m, 3''-***H***H'), 1.89 – 1.72 (2H, m, 4''-$\boldsymbol{H}_2$), 1.53 – 1.43 (1H, m, 3''-H***H***'); δ_C (101 MHz, $CDCl_3$, mixture of diastereomers) 171.6(8) (***C***=O), 171.6(6) (***C***=O), 139.1(2) (Ar***C***), 139.0(8) (Ar***C***), 138.1 (Ar***C***), 138.0 (Ar***C***), 133.2 (***C***-4'), 130.4 (Ar***C***), 130.3 (Ar***C***), 129.0 (Ar***C***), 128.9(3) (Ar***C***), 128.8(8) (Ar***C***), 128.8(5) (Ar***C***), 128.8 (Ar***C***), 127.6 (Ar***C***), 77.7 (***C***-2''), 77.6 (***C***-2''), 68.3 (***C***-5''), 58.6 (***C***-2), 58.5 (***C***-2), 43.4(2) (NH***C***HH' isomer B), 43.4(1) (NH***C***HH' isomer A), 28.6(2) (***C***-3''), 28.6(0) (***C***-3''), 26.0 (***C***-4''); *m/z* (LCMS, ESI^+) 330 ($M(^{35}Cl)H^+$), 332 ($M(^{37}Cl)H^+$); Accurate mass: Found MH^+, 330.1261: $C_{19}H_{21}NO_2{}^{35}Cl$ requires *M*, 330.1261.

***N*-(3''-[2'''-Pyrrolidinon-1'''-yl]prop-1''-yl)-2-(4'-chlorophenyl)-2-phenylacetamide 134**



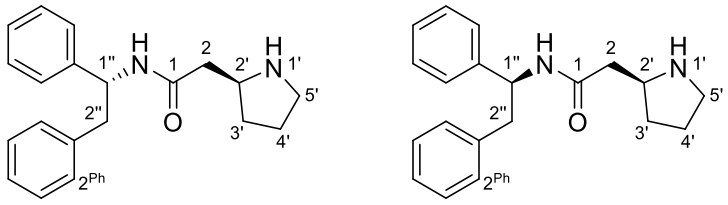
Procedure: Acid **90** (50 mg, 0.20 mmol) and 3-(2'-pyrrolidinon-1'-yl)propylamine (28 mg, 0.20 mmol) were reacted according to general procedure A. Following reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (38 mg, 51%) was isolated as a clear colourless oil.

ν_{max} (ATR) 3282 (br), 3052 (w), 2963 (w), 2934 (w), 2880 (w), 1655 (br, s), 1545 (w), 1490 (m), 1428 (w), 1291 (w), 1222 (w), 1089 (w) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.33 – 7.21 (9H, m, Ar***H***), 7.08 – 7.00 (1H, m, N***H***), 4.82 (1H, s, 2-***H***), 3.33 (2H, apparent ddd, *J* = 8.5, 7.0, 1.5 Hz, 5'''-$\boldsymbol{H}_2$), 3.23 – 3.16 (4H, m, 1''-$\boldsymbol{H}_2$, 3''-$\boldsymbol{H}_2$), 2.34 (2H, t, *J* = 8.0 Hz, 3'''-$\boldsymbol{H}_2$), 1.98 (2H, apparent pd, *J* = 7.5, 2.0 Hz, 4'''-$\boldsymbol{H}_2$), 1.64 (2H, p, *J* = 6.0 Hz, 2''-$\boldsymbol{H}_2$); δ_C (101 MHz, $CDCl_3$) 176.1 (***C***-2'''), 171.6 (***C***=O), 139.4 (***C***-1^{Ph}), 138.3 (***C***-1'), 133.0 (***C***-4'), 130.3 (Ar***C***), 128.8 (Ar***C***), 128.7 (Ar***C***), 127.3 (Ar***C***), 58.3 (***C***-2),

47.5 (***C***-5‴), 39.6 (Al***C***), 36.1 (Al***C***), 31.0 (***C***-3‴), 26.5 (***C***-2″), 18.0 (***C***-4‴); *m/z* (LCMS, ESI^+) 371 ($M(^{35}Cl)H^+$), 373 ($M(^{37}Cl)H^+$); Accurate mass: Found MH^+, 371.1516: $C_{21}H_{24}N_2O_2{}^{35}Cl$ requires *M*, 371.1526.

_N_-(1″,2″-Diphenylethyl)-2-(pyrrolidin-2′-yl)acetamide 135

A mixture of (2′*S*,1″*R*) and (2′*S*,1″*S*) diastereomers



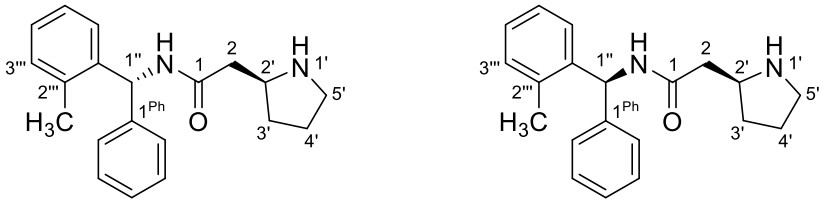
Procedure: *S*-*N*-Boc-homoproline **(*S*)-62** (46 mg, 0.20 mmol) and 1,2-diphenylethylamine (97%, 41 mg, 0.20 mmol) were reacted according to general procedure A. Following TFA-mediated deprotection and reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (32 mg, 52%) was isolated as a yellow oil.

ν_{max} (ATR) 3270 (br), 3094 (w), 3022 (w), 2918 (w), 1670 (s), 1544 (m), 1496 (w), 1199 (s), 1177 (s), 1129 (s), 1028 (w) cm^{-1}; δ_H (700 MHz, $CDCl_3$, mixture of diastereomers) 8.20 (0.5H, d, *J* = 8.0 Hz, 1-N***H*** isomer A), 7.88 (0.5H, d, *J* = 8.0 Hz, 1-N***H*** isomer B), 7.29 – 7.15 (8H, m, Ar***H***), 7.13 (1H, d, *J* = 7.5 Hz, 2^{Ph}-$\boldsymbol{H}_2$ isomer A), 7.09 (1H, d, *J* = 7.0 Hz, 2^{Ph}-$\boldsymbol{H}_2$ isomer B), 5.19 (0.5H, q, *J* = 8.0 Hz, 1″-***H*** isomer A), 5.10 (0.5H, td, *J* = 8.0, 6.5 Hz, 1″-***H*** isomer B), 3.61 – 3.53 (1H, m, 2′-***H***), 3.10 – 3.03 (1H, m, 5′-***H***H′), 3.05 (1H, d, *J* = 8.0 Hz, 2″-$\boldsymbol{H}_2$ isomer A), 3.02 (0.5H, d, *J* = 6.5 Hz, 2″-***H***H′ isomer B), 3.02 (0.5H, d, *J* = 8.0 Hz, 2″-H***H***′ isomer B), 3.00 – 2.95 (1H, m, 5′-H***H***′), 2.77 (0.5H, dd, *J* = 15.5, 7.5 Hz, 2-***H***H′ isomer B), 2.68 (0.5H, dd, *J* = 15.0, 7.0 Hz, 2-***H***H′ isomer A), 2.51 (0.5H, dd, *J* = 15.0, 5.0 Hz, 2-H***H***′ isomer A), 2.49 (0.5H, dd, *J* = 15.5, 5.0 Hz, 2-H***H***′ isomer B), 1.94 – 1.83 (2H, m, 4′-***H***H′ isomer A, 4′-***H***H′ isomer B, 3′-***H***H′ isomer A, 3′-***H***H′ isomer B), 1.81 – 1.72 (1H, m, 4′-H***H***′ isomer A, 4′-H***H***′ isomer B), 1.49 (0.5H, dq, *J* = 12.5, 9.0 Hz, 3′-H***H***′ isomer B), 1.40 (0.5H, dq, *J* = 13.5, 9.5, 8.5 Hz, 3′-H***H***′ isomer A); δ_C (176 MHz, $CDCl_3$, mixture of diastereomers) 169.6(1) (***C***=O isomer A), 169.5(8) (***C***=O isomer B), 141.9 (Ar***C***), 141.7

(Ar***C***), 138.1 (Ar***C***), 137.8 (Ar***C***), 129.5 (***C***-2^{Ph} isomer A), 129.4 (***C***-2^{Ph} isomer B), 128.7(0) (Ar***C***), 128.6(7) (Ar***C***), 128.5 (Ar***C***), 128.4 (Ar***C***), 127.6 (Ar***C***), 127.5 (Ar***C***), 126.8 (Ar***C***), 126.7 (Ar***C***), 126.5(9) (Ar***C***), 126.5(8) (Ar***C***), 57.5 (***C***-2′ isomer A), 57.0 (***C***-2′ isomer B), 55.4 (***C***-1′′ isomer B), 55.0 (***C***-1′′ isomer A), 45.0(0) (***C***-5′), 44.9(6) (***C***-5′), 43.0 (***C***-2′′ isomer B), 42.7 (***C***-2′′ isomer A), 36.9 (***C***-2 isomer B), 36.5 (***C***-2 isomer A), 29.9 (***C***-3′ isomer B), 29.4 (***C***-3′ isomer A), 23.7(3) (***C***-4′), 23.7(0) (***C***-4′); *m/z* (LCMS, ESI$^+$) 309 (MH$^+$); Accurate mass: Found MH$^+$, 309.1961: $C_{20}H_{25}N_2O$ requires *M*, 309.1967.

_N_-([2‴-Methylphenyl]phenylmethyl)-2-(pyrrolidin-2′-yl)acetamide 136

A mixture of (2′*S*,1′′*R*) and (2′*S*,1′′*S*) diastereomers



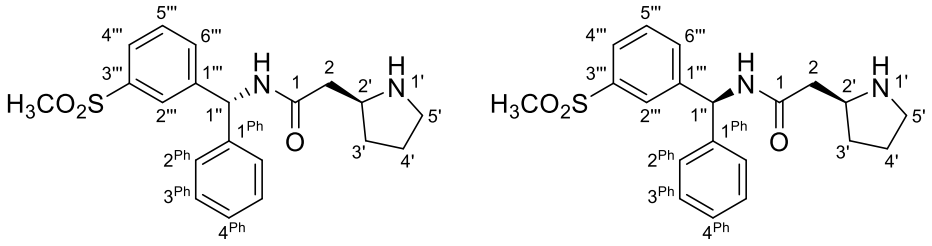
Procedure: *S*-*N*-Boc-homoproline **(*S*)-62** (46 mg, 0.20 mmol) and (2-methylphenyl) phenylmethylamine·HCl (95%, 49 mg, 0.20 mmol) were reacted according to general procedure A. Following TFA-mediated deprotection and reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (45 mg, 73%) was isolated as a white solid.

M.p. 178 – 181 °C; ν_{max} (ATR) 3254 (w), 3060 (w), 3022 (w), 1674 (s), 1632 (s), 1558 (s), 1200 (s), 1188 (m), 1142 (w) cm^{-1}; δ_H (700 MHz, $CDCl_3$, mixture of diastereomers) 7.31 – 7.27 (2H, m, Ar***H***) 7.26 – 7.22 (2H, m, Ar***H***), 7.21 – 7.10 (5H, m, Ar***H***), 6.30 (0.5H, d, *J* = 7.5 Hz, 1′′-***H***), 6.26 (0.5H, d, *J* = 7.5 Hz, 1′′-***H***), 3.80 – 3.74 (1H, m, 2′-***H***), 3.17 – 3.07 (2H, m, 5′-***H***$_2$), 2.91 (0.5H, dd, *J* = 16.5, 7.5 Hz, 2-***H***H′ isomer A), 2.88 (0.5H, dd, *J* = 16.5, 7.5 Hz, 2-***H***H′ isomer B), 2.76 (0.5H, dd, *J* = 16.5, 5.0 Hz, 2-H***H***′ isomer B), 2.73 (0.5H, dd, *J* = 16.5, 4.5 Hz, 2-H***H***′ isomer A), 2.27 (1.5H, s, 2‴-C***H***$_3$), 2.22 (1.5H, s, 2‴-C***H***$_3$), 2.10 – 2.04 (1H, m, 3′-***H***H′), 2.00 – 1.91 (1H, m, 4′-***H***H′), 1.91 – 1.82 (1H, m, 4′-H***H***′), 1.71 – 1.61 (1H, m, 3′-H***H***′); δ_C (176 MHz, $CDCl_3$, mixture of

diastereomers) 169.6(0) (***C***-1), 169.5(8) (***C***-1), 140.5 (***C***-1^{Ph}), 140.3 (***C***-1^{Ph}), 139.2 (Ar***C***), 139.0 (Ar***C***), 136.1 (Ar***C***), 136.0 (Ar***C***), 130.9(2) (***C***-3‴), 130.9(0) (***C***-3‴), 128.9(3) (Ar***C***), 128.8(6) (Ar***C***), 127.8(4) (Ar***C***), 127.7(9) (Ar***C***), 127.7(5) (Ar***C***), 127.7 (Ar***C***), 127.5 (Ar***C***), 127.0 (Ar***C***), 126.6 (Ar***C***), 126.5(3) (Ar***C***), 126.4(9) (Ar***C***), 56.9(0) (***C***-2′), 56.8(5) (***C***-2′), 54.8 (***C***-1″), 54.5 (***C***-1″), 45.0(0) (***C***-5′), 44.9(8) (***C***-5′), 36.4 (***C***-2 isomer A), 36.2 (***C***-2 isomer B), 30.0 (***C***-3′), 29.9 (***C***-3′), 23.9(2) (***C***-4′), 23.9(1) (***C***-4′), 19.6 (2‴-***C***H_3), 19.5 (2‴-***C***H_3); *m/z* (LCMS, ESI^+) 309 (MH^+); Accurate mass: Found MH^+, 309.1978: $C_{20}H_{25}N_2O$ requires *M*, 309.1967.

_N_-([3‴-{Methanesulfonyl}phenyl]phenylmethyl)-2-(pyrrolidin-2′-yl)acetamide 137

A mixture of (2′*S*,1″*R*) and (2′*S*,1″*S*) diastereomers



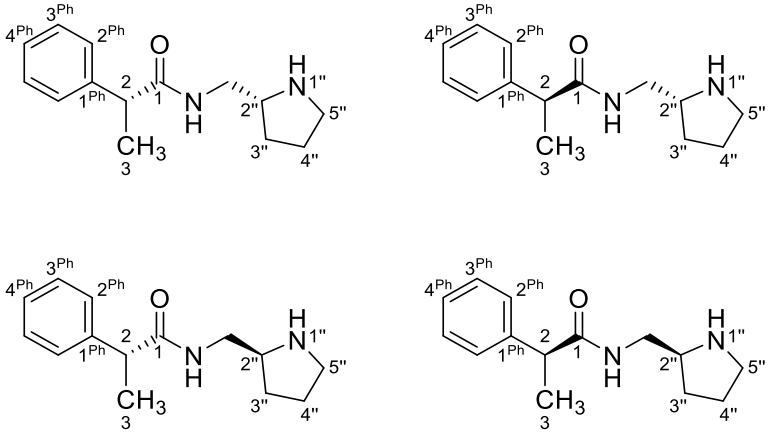
Procedure: *S*-*N*-Boc-homoproline **(*S*)-62** (46 mg, 0.20 mmol) and (3-[methanesulfonyl] phenyl)phenyl methylamine (95%, 55 mg, 0.20 mmol) were reacted according to general procedure A. Following TFA-mediated deprotection and reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (40 mg, 53%) was isolated as a yellow oil.

ν_{max} (ATR) 3300 (br), 3038 (w), 2964 (w), 2866 (w), 1658 (m), 1532 (m), 1418 (w), 1302 (s), 1146 (s), 1092 (w) cm^{-1}; δ_H (700 MHz, $CDCl_3$, mixture of diastereomers) 9.14 (0.5H, d, *J* = 8.0 Hz, 1-N***H*** isomer B), 9.10 (0.5H, d, *J* = 8.0 Hz, 1-N***H*** isomer A), 7.92 (0.5H, t, *J* = 2.0 Hz, 2‴-***H***), 7.87 (0.5H, t, *J* = 2.0 Hz, 2‴-***H***), 7.82 – 7.78 (1H, m, 4‴-***H***), 7.54 – 7.51 (1H, m, 6‴-***H***), 7.51 – 7.47 (1H, m, 5‴-***H***), 7.34 – 7.29 (2H, m, 3^{Ph}-$\boldsymbol{H}_2$), 7.28 – 7.25 (1H, m, 4^{Ph}-***H***), 7.22 – 7.18 (2H, m, 2^{Ph}-$\boldsymbol{H}_2$), 6.30 (1H, d, *J* = 8.0 Hz, 1″-***H***), 3.45 (0.5H, ddt, *J* = 8.0, 7.5, 3.5 Hz, 2′-***H*** isomer A), 3.40 (0.5H, ddt, *J* = 8.5, 7.5, 3.5 Hz, 2′-***H*** isomer B), 3.02 (1.5H, s, $SO_2C\boldsymbol{H}_3$), 3.01 (1.5H, s, $SO_2C\boldsymbol{H}_3$), 2.92 (0.5H, ddd, *J* = 11.0, 7.5, 6.0 Hz, 5′-***H***H′ isomer B), 2.89 (0.5H, ddd, *J* = 11.0, 8.0, 6.0 Hz, 5′-H***H***′

isomer B), 2.87 (0.5H, ddd, *J* = 11.0, 8.0, 6.5 Hz, 5′-***H***H′ isomer A), 2.79 (0.5H, ddd, *J* = 11.0, 7.5, 6.5 Hz, 5′-H***H***′ isomer A), 2.47 (0.5H, dd, *J* = 15.5, 3.5 Hz, 2-***H***H′ isomer A), 2.46 (0.5H, dd, *J* = 15.5, 3.5 Hz, 2-***H***H′ isomer B), 2.32 (0.5H, dd, *J* = 15.5, 8.5 Hz, 2-H***H***′ isomer B), 2.30 (0.5H, dd, *J* = 15.5, 8.0 Hz, 2-H***H***′ isomer A), 1.92 – 1.86 (1H, m, 3′-***H***H′ isomer A, 3′-***H***H′ isomer B), 1.80 – 1.73 (0.5H, m, 4′-***H***H′ isomer B), 1.72 – 1.67 (0.5H, m, 4′-H***H***′ isomer B), 1.67 – 1.60 (1H, m, 4′-$\boldsymbol{H}_2$ isomer A), 1.42 – 1.34 (0.5H, m, 3′-H***H***′ isomer B), 1.38 – 1.30 (0.5H, m, 3′-H***H***′ isomer A); δ_C (176 MHz, $CDCl_3$, mixture of diastereomers) 171.7 (***C***-1 isomer A), 171.6 (***C***-1 isomer B), 144.5 (***C***-1‴), 144.4 (***C***-1‴), 141.2 (***C***-1^{Ph} isomer A), 141.1 (***C***-1^{Ph} isomer B), 141.0 (***C***-3‴), 140.9 (***C***-3‴), 132.9(1) (***C***-6‴), 132.8(6) (***C***-6‴), 129.7 (***C***-5‴), 129.6 (***C***-5‴), 129.1 (***C***-3^{Ph}), 129.0 (***C***-3^{Ph}), 128.0 (***C***-4^{Ph}), 127.9 (***C***-4^{Ph}), 127.7 (***C***-2^{Ph}), 127.5 (***C***-2^{Ph}), 126.2 (***C***-4‴), 126.1 (***C***-4‴), 125.7 (***C***-2‴), 125.5 (***C***-2‴), 56.4 (***C***-1″), 55.7(2) (***C***-2′), 55.6(9) (***C***-2′), 46.2 (***C***-5′ isomer B), 46.1 (***C***-5′ isomer A), 44.5(7) ($SO_2\boldsymbol{C}H_3$), 44.5(6) ($SO_2\boldsymbol{C}H_3$), 40.8 (***C***-2 isomer A), 40.7 (***C***-2 isomer B), 31.4 (***C***-3′ isomer B), 31.2 (***C***-3′ isomer A), 25.3(9) (***C***-4′ isomer A), 25.3(6) (***C***-4′ isomer B); *m/z* (LCMS, ESI^+) 373 (MH^+); Accurate mass: Found MH^+, 373.1592: $C_{20}H_{25}N_2O_3S$ requires *M*, 373.1586.

_N_-([Pyrrolidin-2″-yl]methyl)-2-phenylpropionamide 138

A mixture of (2″*R*,2*R*), (2″*R*,2*S*), (2″*S*,2*R*) and (2″*S*,2*S*) diastereomers

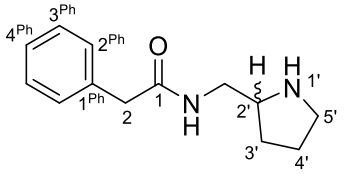


Procedure: 2-Phenylpropionic acid (97%, 31 mg, 0.20 mmol) and 2-(aminomethyl)-1-Boc-pyrrolidine **91** (40 mg, 0.20 mmol) were reacted according to general procedure A. Following

TFA-mediated deprotection and reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (13 mg, 28%) was isolated as a yellow oil.

ν_{max} (ATR) 3282 (br), 3058 (w), 3020 (w), 2964 (w), 2876 (w), 1647 (s), 1542 (br, m), 1456 (w), 1408 (w), 1360 (w), 1234 (w), 1184 (w), 1118 (w), 1070 (w) cm^{-1}; δ_H (600 MHz, $CDCl_3$, mixture of diastereomers) 7.35 – 7.28 (4H, m, 2^{Ph}-***H***$_2$, 3^{Ph}-***H***$_2$), 7.26 – 7.22 (1H, m, 4^{Ph}-***H***), 6.01 – 5.87 (1H, m, N***H***), 3.55 (0.5H, q, *J* = 7.0 Hz, 2-***H*** isomer A), 3.54 (0.5H, q, *J* = 7.0 Hz, 2-***H*** isomer B), 3.31 (0.5H, ddd, *J* = 13.5, 6.5, 4.5 Hz, NHC***H***H′ isomer A), 3.30 (0.5H, ddd, *J* = 13.0, 6.5, 4.5 Hz, NHC***H***H′ isomer B), 3.20 (0.5H, qd, *J* = 7.5, 4.5 Hz, 2′′-***H*** isomer B), 3.15 (0.5H, tdd, *J* = 7.5, 7.0, 4.5 Hz, 2′′-***H*** isomer A), 3.05 – 2.97 (1H, m, NHCH***H***′ isomer A, NHCH***H***′ isomer B), 2.85 – 2.76 (1.5H, m, 5′′-***H***$_2$ isomer A, 5′′-***H***H′ isomer B), 2.74 (0.5H, dt, *J* = 10.5, 7.0 Hz, 5′′-H***H***′ isomer B), 1.81 – 1.72 (1H, m, 3′′-***H***H′ isomer A, 3′′-***H***H′ isomer B), 1.72 – 1.63 (0.5H, m, 4′′-***H***H′ isomer A), 1.64 – 1.57 (1.5H, m, 4′′-H***H***′ isomer A, 4′′-***H***$_2$ isomer B), 1.51 (3H, d, *J* = 7.0 Hz, 3-***H***$_3$), 1.28 (0.5H, ddt, *J* = 13.5, 8.5, 7.0 Hz, 3′′-H***H***′ isomer A), 1.27 – 1.18 (0.5H, m, 3′′-H***H***′ isomer B); δ_C (151 MHz, $CDCl_3$, mixture of diastereomers) 174.5 (***C***=O isomer A), 174.4 (***C***=O isomer B), 141.7(7) (***C***-1^{Ph} isomer B), 141.7(5) (***C***-1^{Ph} isomer A), 128.9 (***C***-3^{Ph}), 127.7(0) (***C***-2^{Ph}), 127.6(9) (***C***-2^{Ph}), 127.3 (***C***-4^{Ph}), 57.6(7) (***C***-2′′ isomer A), 57.6(5) (***C***-2′′ isomer B), 47.3(3) (***C***-2), 47.3(1) (***C***-2), 46.7 (***C***-5′′ isomer B), 46.6 (***C***-5′′ isomer A), 44.0 (NH***C***HH′ isomer A), 43.9 (NH***C***HH′ isomer B), 29.1 (***C***-3′′ isomer A), 29.0 (***C***-3′′ isomer B), 26.0 (***C***-4′′), 18.7 (***C***-3), 18.6 (***C***-3); *m/z* (LCMS, ESI^+) 233 (MH^+); Accurate mass: Found MH^+, 233.1654: $C_{14}H_{21}N_2O$ requires *M*, 233.1654.

_N_-([Pyrrolidin-2′-yl]methyl)-2-phenylacetamide[293] 139



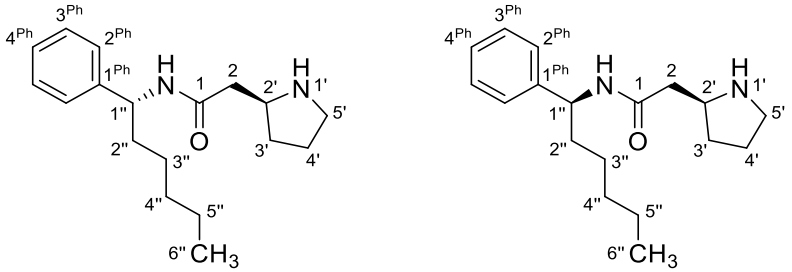
Procedure: 2-Phenylacetic acid (27 mg, 0.20 mmol) and 2-(aminomethyl)-1-Boc-pyrrolidine **91** (40 mg, 0.20 mmol) were reacted according to general procedure A. Following TFA-mediated

deprotection and reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the title compound (7 mg, 16%) was isolated as a clear colourless oil.

ν_{max} (ATR) 3014 (br), 2892 (w), 1674 (s), 1558 (w), 1444 (w), 1364 (s), 1201 (s), 1128 (w) cm^{-1}; δ_H (600 MHz, $CDCl_3$) 7.82 – 7.77 (1H, m, N***H***), 7.31 (2H, t, *J* = 7.5 Hz, 3^{Ph}-***H***$_2$), 7.26 – 7.25 (1H, m, 4^{Ph}-***H***), 7.23 (2H, d, *J* = 7.5 Hz, 2^{Ph}-***H***$_2$), 3.77 – 3.71 (1H, m, 2′-***H***), 3.56 (1H, apparent dd, *J* = 15.0, 7.5 Hz, NHC***H***H′), 3.53 (2H, s, 2-***H***$_2$), 3.37 (1H, dt, *J* = 15.0, 3.5 Hz, NHCH***H***′), 3.15 (1H, ddd, *J* = 11.5, 8.0, 6.0 Hz, 5′-***H***H′), 3.07 (1H, dt, *J* = 11.5, 7.5 Hz, 5′-H***H***′), 2.09 – 2.00 (1H, m, 3′-***H***H′), 1.99 – 1.87 (2H, m, 4′-***H***$_2$), 1.62 (1H, dq, *J* = 13.0, 8.5 Hz, 3′-H***H***′); δ_C (151 MHz, $CDCl_3$) 173.8 (***C***=O), 134.8 (***C***-1^{Ph}), 129.5 (***C***-2^{Ph}), 129.0 (***C***-3^{Ph}), 127.5 (***C***-4^{Ph}), 60.3 (***C***-2′), 45.2 (***C***-5′), 43.2 (***C***-2), 41.0 (NH***C***HH′), 27.8 (***C***-3′), 24.1 (***C***-4′); *m/z* (LCMS, ESI^+) 219 (MH^+); Accurate mass: Found MH^+, 219.1497: $C_{13}H_{19}N_2O$ requires *M*, 219.1497.

_N_-(1″-Phenylhexyl)-2-(pyrrolidin-2′-yl)acetamide 140

A mixture of (2′*S*,1″*R*) and (2′*S*,1″*S*) diastereomers



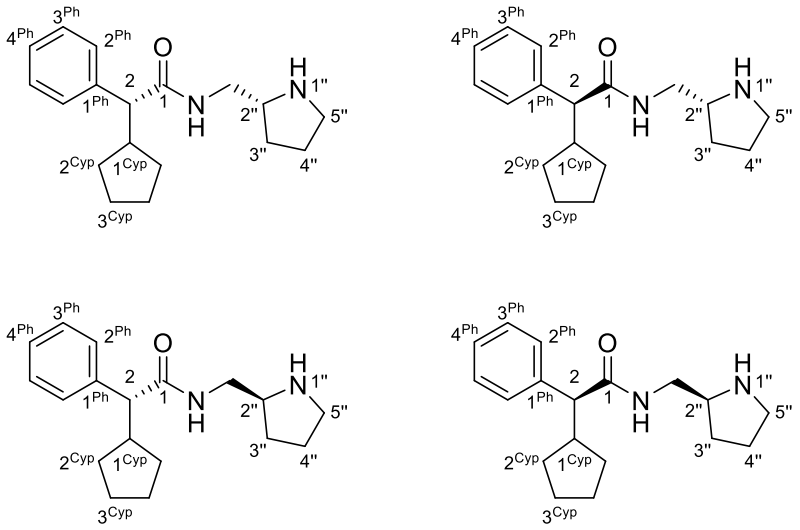
Procedure: *S*-*N*-Boc-homoproline **(*S*)-62** (46 mg, 0.20 mmol) and 1-phenylhexylamine (95%, 37 mg, 0.20 mmol) were reacted according to general procedure A. Following TFA-mediated deprotection and reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (18 mg, 31%) was isolated as a yellow oil.

ν_{max} (ATR) 3290 (br), 3076 (w), 2964 (w), 2930 (w), 2858 (w), 1650 (s), 1558 (m), 1456 (w), 1418 (w), 1199 (s), 1175 (s), 1130 (s) cm^{-1}; δ_H (700 MHz, $CDCl_3$, mixture of diastereomers) 7.74 (0.5H, d, *J* = 7.5 Hz, 1-N***H*** isomer A), 7.68 (0.5H, d, *J* = 7.5 Hz, 1-N***H*** isomer B), 7.31 – 7.27 (2H, m, 3^{Ph}-***H***$_2$), 7.26 – 7.23 (2H, m, 2^{Ph}-***H***$_2$), 7.23 – 7.19 (1H, m, 4^{Ph}-***H***), 4.78 (0.5H, q, *J* = 7.5 Hz, 1″-***H***

isomer B), 4.76 (0.5H, q, *J* = 7.5 Hz, 1''-***H*** isomer A), 3.79 – 3.72 (1H, m, 2'-***H***), 3.21 – 3.15 (1H, m, 5'-***H***H'), 3.13 – 3.07 (1H, m, 5'-H***H***'), 2.86 (0.5H, dd, *J* = 16.0, 7.5 Hz, 2-***H***H' isomer B), 2.81 (0.5H, dd, *J* = 15.5, 7.0 Hz, 2-***H***H' isomer A), 2.65 (0.5H, dd, *J* = 16.0, 5.0 Hz, 2-H***H***' isomer B), 2.62 (0.5H, dd, *J* = 15.5, 5.0 Hz, 2-H***H***' isomer A), 2.11 – 2.04 (1H, m, 3'-***H***H' isomer A, 3'-***H***H' isomer B), 2.02 – 1.94 (1H, m, 4'-***H***H'), 1.92 – 1.83 (1H, m, 4'-H***H***'), 1.80 – 1.60 (3H, m, 2''-$\boldsymbol{H}_2$, 3'-H***H***' isomer A, 3'-H***H***' isomer B), 1.32 – 1.13 (6H, m, 3''-$\boldsymbol{H}_2$, 4''-$\boldsymbol{H}_2$, 5''-$\boldsymbol{H}_2$), 0.88 – 0.80 (3H, m, 6''-$\boldsymbol{H}_3$); δ_C (176 MHz, $CDCl_3$, mixture of diastereomers) 169.8 (***C***=O isomer B), 169.7 (***C***=O isomer A), 142.7 (***C***-1^{Ph} isomer B), 142.5 (***C***-1^{Ph} isomer A), 128.7 (***C***-3^{Ph}), 127.4 (***C***-4^{Ph}), 127.3 (***C***-4^{Ph}), 126.7 (***C***-2^{Ph} isomer A), 126.5 (***C***-2^{Ph} isomer B), 57.0(9) (***C***-2' isomer A), 57.0(5) (***C***-2' isomer B), 54.4(4) (***C***-1'' isomer A), 54.3(6) (***C***-1'' isomer B), 45.0 (***C***-5' isomer B), 44.9 (***C***-5' isomer A), 36.9 (***C***-2 isomer B), 36.6(4) (***C***-2'' isomer B), 36.6(2) (***C***-2 isomer A), 36.3 (***C***-2'' isomer A), 31.6(4) (***C***-4''), 31.6(1) (***C***-4''), 30.1 (***C***-3' isomer B), 29.8 (***C***-3' isomer A), 26.0(9) (***C***-3'' isomer B), 26.0(6) (***C***-3'' isomer A), 24.0 (***C***-4' isomer A), 23.9 (***C***-4' isomer B), 22.6(1) (***C***-5''), 22.5(9) (***C***-5''), 14.1(3) (***C***-6''), 14.1(2) (***C***-6''); *m/z* (LCMS, ESI^+) 289 (MH^+); Accurate mass: Found MH^+, 289.2284: $C_{18}H_{29}N_2O$ requires *M*, 289.2280.

***N*-([Pyrrolidin-2′′-yl]methyl)-2-(phenyl)-2-cyclopentylacetamide 141**

A mixture of (2′′*R*,2*R*), (2′′*R*,2*S*), (2′′*S*,2*R*) and (2′′*S*,2*S*) diastereomers

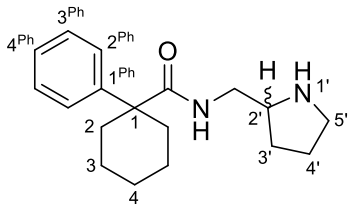


Procedure: 2-Phenyl-2-cyclopentylacetic acid (41 mg, 0.20 mmol) and 2-(aminomethyl)-1-Boc-pyrrolidine **91** (40 mg, 0.20 mmol) were reacted according to general procedure A. Following TFA-mediated deprotection and reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (6 mg, 11%) was isolated as a light yellow oil.

ν_{max} (ATR) 3296 (br), 2950 (m), 2868 (w), 1645 (s), 1542 (m), 1446 (w), 1374 (w), 1222 (w) cm^{-1}; δ_H (600 MHz, $CDCl_3$, mixture of diastereomers) 7.38 – 7.31 (2H, m, 2^{Ph}-***H***$_2$), 7.28 (2H, td, *J* = 7.5, 2.5 Hz, 3^{Ph}-***H***$_2$), 7.24 – 7.20 (1H, m, 4^{Ph}-***H***), 6.21 – 6.11 (1H, m, N***H***), 3.37 (0.5H, ddd, *J* = 13.5, 6.0, 4.5 Hz, NHC***H***H′ isomer A), 3.29 (0.5H, ddd, *J* = 13.0, 6.0, 4.0 Hz, NHC***H***H′ isomer B), 3.22 (0.5H, qd, *J* = 7.5, 4.5 Hz, 2′′-***H*** isomer A), 3.17 (0.5H, ddt, *J* = 7.5, 7.0, 4.0 Hz, 2′′-***H*** isomer B), 3.06 (0.5H, ddd, *J* = 13.0, 7.5, 5.5 Hz, NHCH***H***′ isomer B), 2.99 (1H, d, *J* = 11.0 Hz, 2-***H***), 2.96 (0.5H, ddd, *J* = 13.5, 7.5, 5.0 Hz, NHCH***H***′ isomer A), 2.88 – 2.81 (1.5H, m, 5′′-***H***H′ isomer A, 5′′-***H***$_2$ isomer B), 2.78 (0.5H, dt, *J* = 10.5, 6.5 Hz, 5′′-H***H***′ isomer A), 2.67 – 2.56 (1H, m, 1^{Cyp}-***H***), 1.98 – 1.91 (1H, m, 2^{Cyp}-(***H***H′)(HH′)), 1.82 – 1.73 (1H, m, 3′′-***H***H′ isomer A, 3′′-***H***H′ isomer B), 1.73 – 1.68 (0.5H, m, 4′′-***H***H′ isomer B), 1.68 – 1.51 (4.5H, m, 3^{Cyp}-(***H***H′)H$_2$, 3^{Cyp}-(HH′)***H***$_2$, 4′′-***H***$_2$ isomer A, 4′′-H***H***′ isomer B), 1.51 – 1.45 (1H, m, 3^{Cyp}-(H***H***′)H$_2$), 1.45 – 1.38 (1H, m, 2^{Cyp}-(HH′)(***H***H′)), 1.31 (0.5H, ddt, *J* = 12.5, 8.5, 7.0 Hz, 3′′-H***H***′ isomer B), 1.28 – 1.19 (1.5H, m, 2^{Cyp}-(H***H***′)(HH′), 3′′-H***H***′

isomer A), 0.98 (1H, dq, *J* = 12.5, 8.5 Hz, 2^{Cyp}-(HH')(H***H***')); δ_C (151 MHz, $CDCl_3$, mixture of diastereomers) 173.8 (***C***=O isomer B), 173.7 (***C***=O isomer A), 140.5(3) (***C***-1^{Ph}), 140.5(2) (***C***-1^{Ph}), 128.6 (***C***-3^{Ph}), 128.1 (***C***-2^{Ph}), 127.1 (***C***-4^{Ph}), 60.2 (***C***-2), 57.9 (***C***-2'' isomer A), 57.7 (***C***-2'' isomer B), 46.6(7) (***C***-5''), 46.6(5) (***C***-5''), 43.7 (NH***C***HH'), 43.4 (***C***-1^{Cyp}), 43.3 (***C***-1^{Cyp}), 31.8(6) (***C***-2^{Cyp}), 31.8(5) (***C***-2^{Cyp}), 31.1 (***C***-2^{Cyp}), 29.0(2) (***C***-3'' isomer A), 28.9(9) (***C***-3'' isomer B), 26.0(2) (***C***-4'' isomer A), 25.9(9) (***C***-4'' isomer B), 25.3(4) (***C***-3^{Cyp}), 25.3(3) (***C***-3^{Cyp}), 25.0(4) (***C***-3^{Cyp}), 25.0(3) (***C***-3^{Cyp}); *m/z* (LCMS, ESI^+) 287 (MH^+); Accurate mass: Found MH^+, 287.2120: $C_{18}H_{27}N_2O$ requires *M*, 287.2123.

***N*-([Pyrrolidin-2'-yl]methyl)-1-phenyl-cyclohexane-1-carboxamide 142**



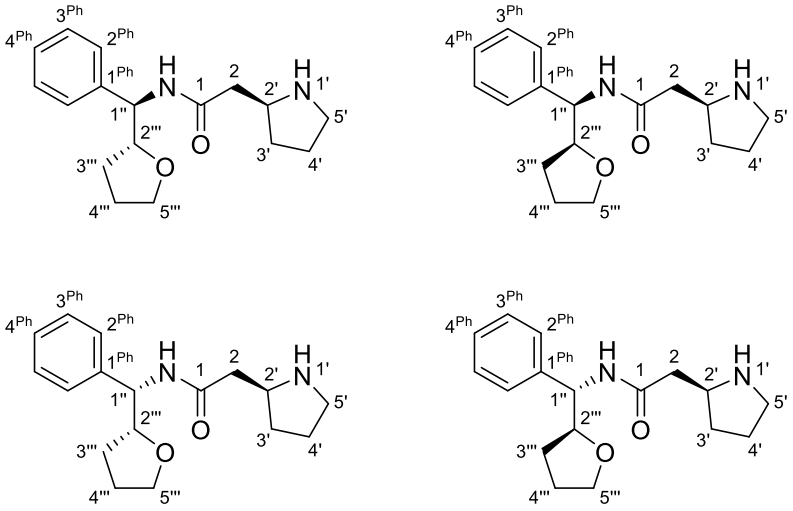
Procedure: 1-Phenylcyclohexane-1-carboxilic acid (41 mg, 0.20 mmol) and 2-(aminomethyl)-1-Boc-pyrrolidine **91** (40 mg, 0.20 mmol) were reacted according to general procedure A. Following TFA-mediated deprotection and reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (33 mg, 58%) was isolated as an orange oil.

ν_{max} (ATR) 3340 (br), 2942 (m), 2856 (w), 2782 (w), 1671 (s), 1594 (s), 1524 (s), 1446 (m), 1199 (s), 1131 (s) cm^{-1}; δ_H (600 MHz, $CDCl_3$) 7.41 (2H, d, *J* = 7.5 Hz, 2^{Ph}-***H***$_2$), 7.34 – 7.28 (1H, m, N***H***), 7.30 (2H, t, *J* = 7.5 Hz, 3^{Ph}-***H***$_2$), 7.20 (1H, t, *J* = 7.5 Hz, 4^{Ph}-***H***), 3.69 – 3.61 (2H, m, NHC***H***H', 2'-***H***), 3.29 – 3.23 (1H, m, NHCH***H***'), 2.99 (1H, ddd, *J* = 11.5, 7.5, 4.5 Hz, 5'-***H***H'), 2.60 (1H, ddd, *J* = 11.5, 9.0, 7.0 Hz, 5'-H***H***'), 2.40 – 2.29 (2H, m, 2-(***H***H')(HH'), 2-(HH')(***H***H')), 2.00 – 1.93 (1H, m, 2-(H***H***')(HH')), 1.93 – 1.82 (2H, m, 3'-***H***H', 2-(HH')(H***H***')), 1.79 – 1.68 (1H, m, 4'-***H***H'), 1.68 – 1.58 (2H, m, 4'-H***H***', 3-(***H***H')H_2), 1.58 – 1.47 (4H, m, 3-(H***H***')H_2, 4-***H***H', 3-(HH')***H***$_2$), 1.43 (1H, ddd, *J* = 16.5, 13.5, 8.0 Hz, 3'-H***H***'), 1.38 – 1.30 (1H, m, 4-H***H***'); δ_C (151 MHz, $CDCl_3$) 177.5 (***C***=O),

144.1 (***C***-1^{Ph}) 128.7 (***C***-3^{Ph}), 126.8 (***C***-4^{Ph}), 126.3 (***C***-2^{Ph}), 59.9 (***C***-2′), 50.6 (***C***-1), 45.2 (***C***-5′), 39.9 (NH***C***HH′), 34.6 (***C***-2), 33.8 (***C***-2), 27.1 (***C***-3′), 25.8 (***C***-4), 24.6 (***C***-4′), 23.3 (***C***-3); *m/z* (LCMS, ESI^+) 287 (MH^+); Accurate mass: Found MH^+, 287.2133: $C_{18}H_{27}N_2O$ requires *M*, 287.2123.

_N_-([Tetrahydrofuran-2‴-yl]phenylmethyl)-2-(pyrrolidin-2′-yl)acetamide 143

A mixture of (2′*S*,1″*R*,2‴*R*), (2′*S*,1″*R*,2‴*S*), (2′*S*,1″*S*,2‴*R*) and (2′*S*,1″*S*,2‴*S*) diastereomers

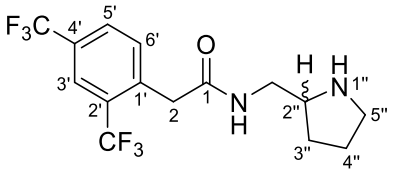


Procedure: *S*-*N*-Boc-homoproline **(*S*)-62** (46 mg, 0.20 mmol) and (tetrahydrofuran-2-yl) phenylmethylamine (95%, 37 mg, 0.20 mmol) were reacted according to general procedure A. Following TFA-mediated deprotection and reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (17 mg, 29%) was isolated as a yellow oil.

ν_{max} (ATR) 3282 (br), 2968 (w), 2876 (w), 1641 (s), 1536 (s), 1070 (m) cm^{-1}; δ_H (600 MHz, $CDCl_3$, mixture of diastereomers) 8.44 (0.3H, d, *J* = 8.5 Hz, 1-N***H***), 8.42 (0.3H, d, *J* = 8.5 Hz, 1-N***H***), 8.22 (0.2H, d, *J* = 8.5 Hz, 1-N***H***), 8.18 (0.2H, d, *J* = 8.5 Hz, 1-N***H***), 7.37 – 7.27 (4H, m, Ar***H***), 7.25 – 7.20 (1H, m, Ar***H***), 5.05 (0.2H, dd, *J* = 8.5, 4.0 Hz, 1″-***H***), 5.04 (0.2H, dd, *J* = 8.5, 4.0 Hz, 1″-***H***), 5.00 (0.3H, dd, *J* = 8.5, 5.0 Hz, 1″-***H***), 4.99 (0.3H, dd, *J* = 8.5, 5.0 Hz, 1″-***H***), 4.22 (0.3H, td, *J* = 7.0, 5.0 Hz, 2‴-***H***), 4.20 (0.3H, td, *J* = 7.0, 5.0 Hz, 2‴-***H***), 4.16 (0.2H, td, *J* = 6.5, 4.0 Hz, 2‴-***H***), 4.14 (0.2H, td, *J* = 6.5, 2.0 Hz, 2‴-***H***), 3.91 (0.2H, td, *J* = 7.0, 3.0 Hz, 5‴-***H***H′), 3.90 (0.2H, td, *J* = 7.0, 3.0 Hz, 5‴-H***H***′), 3.78 (0.2H, td, *J* = 8.0, 6.0 Hz, 5‴-***H***H′), 3.77 (0.2H, td, *J* = 8.0, 6.0 Hz, 5‴-H***H***′),

3.73 – 3.66 (1.2H, m, 5‴-***H***$_2$), 3.42 – 3.31 (1H, m, 2′-***H***), 3.00 (0.3H, ddd, *J* = 11.0, 8.0, 6.0 Hz, 5′-***H***H′), 2.99 (0.3H, ddd, *J* = 10.5, 8.0, 5.5 Hz, 5′-***H***H′), 2.96 – 2.86 (1.4H, m, 5′-H***H***′, 5′-***H***$_2$), 2.46 (0.2H, dd, *J* = 15.0, 4.0 Hz, 2-***H***H′), 2.43 (0.3H, dd, *J* = 15.5, 4.0 Hz, 2-***H***H′), 2.42 (0.2H, dd, *J* = 15.0, 4.0 Hz, 2-H***H***′), 2.39 (0.3H, dd, *J* = 15.5, 4.0 Hz, 2-H***H***′), 2.31 (0.2H, dd, *J* = 15.5, 8.5 Hz, 2-***H***H′), 2.28 (0.2H, dd, *J* = 15.5, 8.5 Hz, 2-H***H***′), 2.27 (0.3H, dd, *J* = 15.0, 8.5 Hz, 2-***H***H′), 2.24 (0.3H, dd, *J* = 15.0, 8.0 Hz, 2-H***H***′), 1.97 – 1.78 (3.4H, m, 4′-***H***$_2$, 2‴-***H***$_2$, 4‴-***H***$_2$, 3‴-***H***$_2$), 1.77 – 1.63 (2.4H, m, 4′-***H***$_2$, 4‴-***H***$_2$, 3‴-***H***$_2$), 1.63 – 1.56 (0.6H, m, 3‴-***H***$_2$), 1.56 – 1.48 (0.6H, m, 4‴-***H***$_2$), 1.44 – 1.26 (1H, m, 3′-***H***$_2$); δ_C (151 MHz, $CDCl_3$, mixture of diastereomers) 171.8 (***C***=O), 171.3 (***C***=O), 141.5(3) (***C***-1^{Ph}), 141.4(6) (***C***-1^{Ph}), 139.6(0) (***C***-1^{Ph}), 139.5(5) (***C***-1^{Ph}), 128.6 (Ar***C***), 128.4 (Ar***C***), 128.3(1) (Ar***C***), 128.2(6) (Ar***C***), 127.5 (Ar***C***), 127.4 (Ar***C***), 127.3(0) (Ar***C***), 127.2(6) (Ar***C***), 127.2 (Ar***C***), 127.1 (Ar***C***), 81.8 (***C***-2‴), 81.7 (***C***-2‴), 81.4(4) (***C***-2‴), 81.4(2) (***C***-2‴), 69.0 (***C***-5‴), 68.9(0) (***C***-5‴), 68.8(5) (***C***-5‴), 68.8 (***C***-5‴), 56.3 (***C***-1″), 56.2 (***C***-1″), 56.0(2) (***C***-2′), 55.9(5) (***C***-2′), 55.9 (***C***-2′), 55.8 (***C***-2′), 55.5(4) (***C***-1″), 55.4(8) (***C***-1″), 46.3(4) (***C***-5′), 46.3(0) (***C***-5′), 46.2 (***C***-5′), 41.6(3) (***C***-2), 41.5(9) (***C***-2), 41.5 (***C***-2), 41.3 (***C***-2), 31.5 (***C***-3′), 31.4 (***C***-3′), 31.3 (***C***-3′), 31.1 (***C***-3′), 29.2(4) (***C***-3‴), 29.1(8) (***C***-3‴), 28.4(3) (***C***-3‴), 28.4(0) (***C***-3‴), 26.0(3) (***C***-4‴), 25.9(8) (***C***-4‴), 25.7(8) (***C***-4‴), 25.7(5) (***C***-4‴), 25.4 (***C***-4′), 25.3 (***C***-4′), 25.2(3) (***C***-4′), 25.1(8) (***C***-4′); *m/z* (LCMS, ESI$^+$) 289 (MH^+); Accurate mass: Found MH^+, 289.1902: $C_{17}H_{25}N_2O_2$ requires *M*, 289.1916.

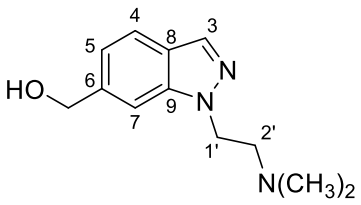
_N_-([Pyrrolidin-2″-yl]methyl)-2-(2′,4′-di[trifluoromethyl]phenyl)acetamide 144



Procedure: 2-(2,4-di[trifluoromethyl]phenyl)acetic acid (54 mg, 0.20 mmol) and 2-(aminomethyl)-1-Boc-pyrrolidine **91** (40 mg, 0.20 mmol) were reacted according to general procedure A. Following TFA-mediated deprotection and reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (35 mg, 49%) was isolated as an off-white solid.

M.p. 90 – 93 °C; ν_{max} (ATR) 3282 (br), 3072 (w), 2964 (w), 2868 (w), 1655 (m), 1564 (w), 1524 (w), 1346 (s), 1276 (s), 1125 (s), 1056 (m) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 7.91 (1H, s, 3′-***H***), 7.79 (1H, d, *J* = 8.0 Hz, 5′-***H***), 7.68 (1H, d, *J* = 8.0 Hz, 6′-***H***), 6.14 (1H, br m, N-***H***), 3.76 (2H, s, 2-$\boldsymbol{H}_2$), 3.38 (1H, ddd, *J* = 13.5, 6.0, 4.5 Hz, NHC***H***H′), 3.26 (1H, dddd, *J* = 8.0, 7.5, 7.0, 4.5 Hz, 2′′-***H***), 3.02 (1H, ddd, *J* = 13.5, 7.5, 5.0 Hz, NHCH***H***′), 2.90 (1H, ddd, *J* = 10.5, 7.5, 6.0 Hz, 5′′-***H***H′), 2.83 (1H, dt, *J* = 10.5, 6.5 Hz, 5′′-H***H***′), 1.93 (1H, br m, 1′′-***H***), 1.83 (1H, dtd, *J* = 13.0, 8.0, 5.5 Hz, 3′′-***H***H′), 1.77 – 1.69 (1H, m, 4′′-***H***H′), 1.72 – 1.63 (1H, m, 4′′-H***H***′), 1.33 (1H, ddt, *J* = 13.0, 9.0, 7.0 Hz, 3′′-H***H***′); δ_C (176 MHz, $CDCl_3$) 168.7 (***C***=O), 137.9 (***C***-1′), 133.6 (***C***-6′), 130.0 (q, *J* = 33.5 Hz, ***C***-4′), 129.62 (q, *J* = 31.0 Hz, ***C***-2′), 128.9 (***C***-5′), 124.53 (q, *J* = 274.0 Hz, 2′-***C***F_3), 123.48 (q, *J* = 272.0 Hz, 4′-***C***F_3), 123.4 (***C***-3′), 57.4 (***C***-2′′), 46.7 (***C***-5′′), 44.1 (NH***C***HH′), 40.1 (***C***-2), 29.2 (***C***-3′′), 26.1 (***C***-4′′); δ_F (376 MHz, $CDCl_3$) −60.40 (Al***F***), −63.30 (Al***F***); *m/z* (LCMS, ESI^+) 355 (MH^+); Accurate mass: Found MH^+, 355.1249: $C_{15}H_{17}N_2OF_6$ requires *M*, 355.1245.

1-(2′-[Dimethylamino]ethyl)-6-(hydroxymethyl)indazole 145

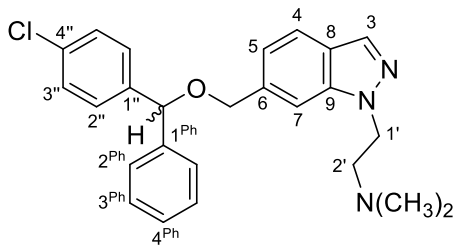


Procedure: To a stirred suspension of $LiAlH_4$ (300 mg, 7.89 mmol) in THF (15 mL) at 0 °C was slowly added a solution of ester **149** (650 mg, 2.63 mmol) in THF (3 mL). After 1 h at RT, the mixture was re-cooled in an ice bath and quenched according to Fieser's method (general procedure D).[273] Subsequent removal of volatiles *in vacuo* afforded the *title compound* (577 mg, quantitative) as a pale yellow oil.

R_f 0.12 (40% EtOAc in $CHCl_3$ with 1% NEt_3); ν_{max} (ATR) 3348 (br), 2974 (m), 2945 (m), 2864 (m), 2829 (m), 2786 (m), 1472 (s), 1372 (m), 1316 (m), 1258 (w), 1208 (m), 1191 (m), 1122 (w), 1044 (s) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 7.96 (1H, d, *J* = 1.0 Hz, 3-***H***), 7.68 (1H, dd, *J* = 8.0, 1.0 Hz, 4-***H***), 7.45 (1H, q, *J* = 1.0 Hz, 7-***H***), 7.11 (1H, dd, *J* = 8.0, 1.0 Hz, 5-***H***), 4.83 (2H, s, C$\boldsymbol{H}_2$OH), 4.46

(2H, t, *J* = 7.0 Hz, 1′-***H***$_2$), 2.81 (2H, t, *J* = 7.0 Hz, 2′-***H***$_2$), 2.28 (6H, s, N(C***H***$_3$)$_2$); δ_C (176 MHz, $CDCl_3$) 140.0 (***C***-6, ***C***-9), 133.2 (***C***-3), 123.6 (***C***-8), 121.4 (***C***-4), 120.1 (***C***-5), 106.8 (***C***-7), 65.5 (***C***H$_2$OH), 58.5 (***C***-2′), 47.2 (***C***-1′), 45.8 (N(***C***H$_3$)$_2$); *m/z* (LCMS, ESI$^+$) 220 (MH^+); Accurate mass: Found MH^+, 220.1441: $C_{12}H_{18}N_3O$ requires *M*, 220.1450.

1-(2′-[Dimethylamino]ethyl)-6-([{4″-chlorophenyl}{phenyl}methoxy]methyl)indazole 146



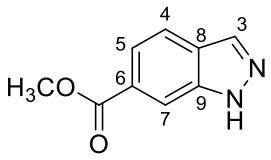
Procedure: To a cooled solution of benzhydryl alcohol **73** (139 mg, 0.64 mmol) in DCM (1 mL) was added NEt_3 (116 µL, 0.83 mmol) and methanesulfonyl chloride (59 µL, 0.76 mmol). The mixture was stirred at RT until consumption of starting material was observed by TLC. After dilution with DCM (10 mL), the mixture was washed with H_2O (5 mL), 1 M $HCl_{(aq.)}$ (5 mL) then saturated $NaHCO_{3(aq.)}$ (5 mL), before being dried over $MgSO_4$. Removal of volatiles under reduced pressure afforded the crude benzhydryl chloride **157** which was used immediately.

To a 0 °C solution of NaH (60%, 18 mg, 0.45 mmol) in DMF (0.25 mL) was added indazole **145** (93 mg, 0.42 mmol) in DMF (0.5 mL). After stirring at RT for 2 h, a solution of crude benzhydryl chloride **157** in DMF (0.5 mL) was added and the reaction mixture heated to 80 °C for 4.5 h. The mixture was re-cooled to 0 °C and quenched by the addition of ice cold H_2O (15 mL) prior to extraction with EtOAc (6 × 5 mL). The combined organic washings were washed with brine (15 mL), concentrated *in vacuo* then purified on SiO_2 column (50% EtOAc in $CHCl_3$ with 1% NEt_3) to furnish the *title compound* (25 mg, 14%) as a clear colourless oil.

R_f 0.28 (40% EtOAc in $CHCl_3$ with 1% NEt_3); ν_{max} (ATR) 3063 (w), 3031 (w), 2971 (w), 2861 (w), 2819 (w), 2768 (w), 1489 (m), 1455 (m), 1366 (s), 1217 (s), 1087 (s), 1014 (m) cm^{-1}; δ_H (600 MHz, $CDCl_3$) 7.98 (1H, s, 3-***H***), 7.69 (1H, d, *J* = 8.0 Hz, 4-***H***), 7.41 (1H, s, 7-***H***), 7.39 – 7.26 (9H, m, Ar***H***), 7.13 (1H, d, *J* = 8.0 Hz, 5-***H***), 5.45 (1H, s, Ar$_2$C***H***), 4.68 (2H, s, OC***H***$_2$), 4.49 (2H, t, *J* = 7.0 Hz, 1′-***H***$_2$), 2.85 (2H, t, *J* = 7.0 Hz, 2′-***H***$_2$), 2.32 (6H, s, N(C***H***$_3$)$_2$); δ_C (151 MHz, $CDCl_3$) 141.7

(***C***-1^{Ph}), 140.8 (***C***-1''), 139.9 (***C***-9), 136.9 (***C***-6), 133.4 (***C***-4''), 133.2 (***C***-3), 128.7(4) (Ar***C***), 128.7(2) (Ar***C***), 128.6 (Ar***C***), 128.0 (Ar***C***), 127.2 (Ar***C***), 123.8 (***C***-8), 121.3 (***C***-4), 120.8 (***C***-5), 107.9 (***C***-7), 82.1 (Ar_2***C***H), 71.0 (O***C***H_2), 58.5 (***C***-2'), 47.1 (***C***-1'), 45.7 (N(***C***$H_3)_2$); *m/z* (LCMS, ESI^+) 420 ($M(^{35}Cl)H^+$), 422 ($M(^{37}Cl)H^+$); Accurate mass: Found MH^+, 420.1845: $C_{25}H_{27}N_3O^{35}Cl$ requires *M*, 420.1843.

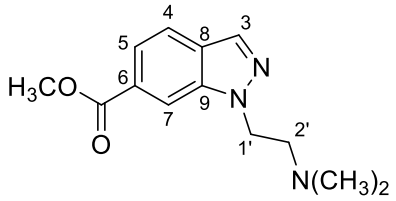
Methyl 1*H*-indazole-6-carboxylate[294] 148



Procedure:[254] A mixture of 1*H*-Indazole-6-carboxylic acid **147** (320 mg, 1.97 mmol) and concentrated H_2SO_4 (0.12 mL) in MeOH (5 mL) was heated to 100 °C for 40 min under microwave irradiation. The reaction mixture was then poured into cold saturated $NaHCO_{3(aq.)}$ solution (20 mL), extracted with EtOAc (3 × 10 mL) and the combined organic fractions washed with brine (20 mL) and dried over $MgSO_4$. Following concentration *in vacuo*, the title compound (309 mg, 89%) was obtained as a yellow solid.

R_f 0.38 (50% EtOAc in hexanes with 1% NEt_3); ν_{max} (ATR) 3017 (w), 2950 (w), 1715 (s), 1435 (w), 1370 (s), 1292 (w), 1218 (s), 1091 (w) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 10.62 (1H, br s N***H***), 8.30 – 8.27 (1H, m, 7-***H***), 8.16 (1H, d, *J* = 1.0 Hz, 3-***H***), 7.85 (1H, dd, *J* = 8.5, 1.5 Hz, 5-***H***), 7.81 (1H, dd, *J* = 8.5, 0.5 Hz, 4-***H***), 3.97 (3H, s, OC***H***$_3$); δ_C (176 MHz, $CDCl_3$) 167.4 (***C***=O), 139.7 (***C***-9), 135.2 (***C***-3), 128.82 (***C***-6), 126.0 (***C***-8), 121.8 (***C***-5), 120.9 (***C***-4), 112.3 (***C***-7), 52.5 (O***C***H_3); *m/z* (LCMS, ESI^+) 177 (MH^+).

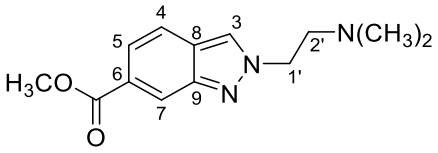
Methyl-1-(2′-[dimethylamino]ethyl)indazole-6-carboxylate 149



Procedure:[295] Methyl ester **148** (264 mg, 1.50 mmol) was stirred with K_2CO_3 (622 mg, 4.50 mmol) and 2-chloro-*N*,*N*-dimethylethylamine hydrochloride salt (99%, 327 mg, 2.25 mmol) in DMF (5 mL) at 80 °C for 14 h. The reaction mixture was then cooled and diluted with H_2O (60 mL) prior to washing with EtOAc (6 × 10 mL). After combining the organic fractions, the volatiles were removed under reduced pressure and the crude mixture separated by flash chromatography on silica (10% → 90% EtOAc in hexanes with 1% NEt_3). Four compounds were isolated, with the *title compound* (199 mg, 54%) found as a yellow oil.

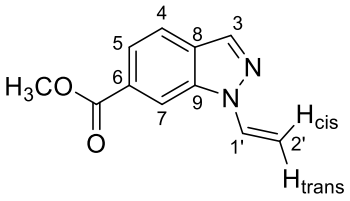
R_f 0.32 (80% EtOAc in hexanes with 1% NEt_3); ν_{max} (ATR) 2980 (w), 2963 (w), 2855 (w), 2820 (w), 1710 (s), 1560 (m), 1501 (m), 1440 (m), 1366 (m), 1327 (w), 1290 (m), 1230 (s), 1152 (m) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 8.19 (1H, q, *J* = 1.0 Hz, 7-***H***), 8.02 (1H, d, *J* = 1.0 Hz, 3-***H***), 7.78 (1H, dd, *J* = 8.5, 1.0 Hz, 5-***H***), 7.73 (1H, dd, *J* = 8.5, 1.0 Hz, 4-***H***), 4.52 (2H, t, *J* = 7.0 Hz, 1′-$\boldsymbol{H}_2$), 3.95 (3H, s, OC$\boldsymbol{H}_3$), 2.83 (2H, t, *J* = 7.0 Hz, 2′-$\boldsymbol{H}_2$), 2.29 (6H, s, N(C$\boldsymbol{H}_3$)$_2$); δ_C (176 MHz, $CDCl_3$) 167.4 (***C***=O), 139.2 (***C***-9), 133.2 (***C***-3), 128.0 (***C***-6), 126.6 (***C***-8), 121.1(0) (***C***-5), 121.0(6) (***C***-4), 111.6 (***C***-7), 58.6 (***C***-2′), 52.4 (O***C***H_3), 47.5 (***C***-1′), 45.8 (N(***C***H_3)$_2$); *m/z* (LCMS, ESI^+) 248 (MH^+); Accurate mass: Found MH^+, 248.1408: $C_{13}H_{18}N_3O_2$ requires *M*, 248.1399.

Further elution afforded **methyl-2-(2′-[dimethylamino]ethyl)indazole-6-carboxylate 150** (90 mg, 24%) as a yellow oil.



R_f 0.13 (80% EtOAc in hexanes with 1% NEt_3); ν_{max} (ATR) 2972 (w), 2950 (w), 2860 (w), 2823 (w), 2773 (w), 1713 (s), 1556 (w), 1501 (m), 1436 (m), 1366 (m), 1328 (m), 1295 (m), 1223 (s), 1148 (m), 1085 (s) cm^{-1}; δ_H (600 MHz, $CDCl_3$) 8.47 (1H, dt, *J* = 1.5, 1.0 Hz, 7-***H***), 8.03 (1H, d, *J* = 1.0 Hz, 3-***H***), 7.67 (1H, dd, *J* = 9.0, 1.5 Hz, 5-***H***), 7.65 (1H, dd, *J* = 9.0, 1.0 Hz, 4-***H***), 4.52 (2H, t, *J* = 6.5 Hz, 1′-$\boldsymbol{H}_2$), 3.92 (3H, s, OC$\boldsymbol{H}_3$), 2.88 (2H, t, *J* = 6.5 Hz, 2′-$\boldsymbol{H}_2$), 2.27 (6H, s, N(C$\boldsymbol{H}_3$)$_2$); δ_C (151 MHz, $CDCl_3$) 167.7 (***C***=O), 148.0 (***C***-9), 127.8 (***C***-6), 124.0 (***C***-8), 123.7 (***C***-3), 121.2(9) (***C***-5), 121.2(6) (***C***-7), 120.3 (***C***-4), 59.2 (***C***-2′), 52.2 (***C***-1′, O***C***H_3), 45.6 (N(***C***H_3)$_2$); *m/z* (LCMS, ESI^+) 248 (MH^+); Accurate mass: Found MH^+, 248.1407: $C_{13}H_{18}N_3O_2$ requires *M*, 248.1399.

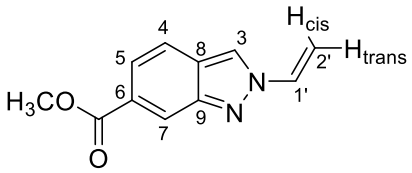
The olefin **methyl-1-vinylindazole-6-carboxylate 151** (38 mg, 13%) was also isolated from this reaction as a pale yellow solid.



R_f 0.74 (80% EtOAc in hexanes with 1% NEt_3); M.p. 81 – 83 °C; ν_{max} (ATR) 3072 (w), 2959 (w), 1710 (s), 1641 (s), 1576 (m), 1472 (m), 1430 (s), 1380 (m), 1342 (s), 1289 (s), 1243 (s), 1214 (s), 1126 (m), 1104 (m) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 8.30 (1H, s, 7-***H***), 8.15 (1H, s, 3-***H***), 7.86 (1H, d, *J* = 8.5 Hz, 5-***H***), 7.77 (1H, d, *J* = 8.5 Hz, 4-***H***), 7.40 (1H, dd, *J* = 15.5, 9.0 Hz, 1′-***H***), 5.79 (1H, d, *J* = 15.5 Hz, 2′-$\boldsymbol{H}_{cis}$), 4.98 (1H, d, *J* = 9.0 Hz, 2′-$\boldsymbol{H}_{trans}$), 3.97 (3H, s, OC$\boldsymbol{H}_3$); δ_C (176 MHz, $CDCl_3$)

167.1 (***C***=O), 138.2 (***C***-9), 135.6 (***C***-3), 129.7 (***C***-1'), 129.1 (***C***-6), 127.5 (***C***-8), 122.5 (***C***-5), 121.3 (***C***-4), 111.7 (***C***-7), 99.8 (***C***-2'), 52.6 (O***C***H_3); *m/z* (LCMS, ESI^+) 203 (MH^+); Accurate mass: Found MH^+, 203.0824: $C_{11}H_{11}N_2O_2$ requires *M*, 203.0821.

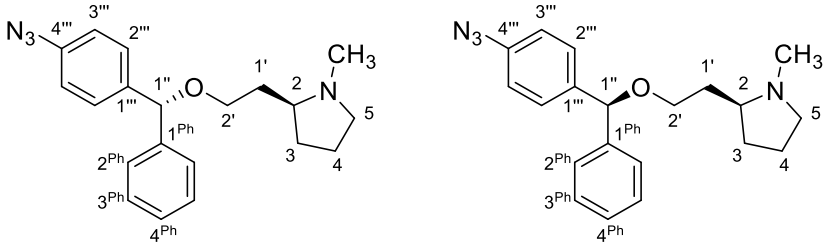
The by-product **methyl-2-vinylindazole-6-carboxylate 152** (15 mg, 5%) was also obtained *via* SiO_2 chromatography as a yellow solid.



R_f 0.61 (80% EtOAc in hexanes with 1% NEt_3); M.p. 86 – 88 °C; ν_{max} (ATR) 3124 (w), 3112 (w), 3001 (w), 2950 (w), 1710 (s), 1646 (m), 1556 (w), 1499 (m), 1434 (m), 1389 (m), 1340 (m), 1300 (m), 1243 (s), 1220 (s), 1154 (m), 1085 (s) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 8.52 – 8.48 (1H, m, 7-***H***), 8.12 (1H, d, *J* = 1.0 Hz, 3-***H***), 7.70 (1H, dd, *J* = 9.0, 1.0 Hz, 5-***H***), 7.67 (1H, dd, *J* = 9.0, 1.0 Hz, 4-***H***), 7.32 (1H, dd, *J* = 15.5, 8.5 Hz, 1'-***H***), 6.02 (1H, dd, *J* = 15.5, 1.5 Hz, 2'-$\boldsymbol{H}_{cis}$), 5.22 (1H, dd, *J* = 8.5, 1.5 Hz, 2'-$\boldsymbol{H}_{trans}$), 3.95 (3H, s, OC$\boldsymbol{H}_3$); δ_C (176 MHz, $CDCl_3$) 167.4 (***C***=O), 148.8 (***C***-9), 133.8 (***C***-1'), 129.1 (***C***-6), 124.1 (***C***-8), 122.3 (***C***-5), 121.8 (***C***-7), 121.4 (***C***-3), 120.5 (***C***-4), 105.9 (***C***-2'), 52.38 (O***C***H_3); *m/z* (LCMS, ESI^+) 203 (MH^+); Accurate mass: Found MH^+, 203.0828: $C_{11}H_{11}N_2O_2$ requires *M*, 203.0821.

2-(2′-[{4‴-Azidophenyl}phenylmethoxy]ethyl)-1-methylpyrrolidine 162

A mixture of (2*S*,1″*R*) and (2*S*,1″*S*) diastereomers



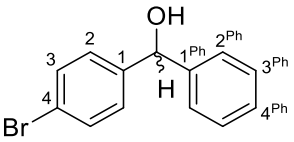
Procedure:[266] Aryl bromide **164** (263 mg, 1 mmol) was dissolved in a degassed mixture of EtOH and H_2O (7 : 3, 2 mL). This solution was then added to a mixture of NaN_3 (130 mg, 2 mmol), CuI (31 mg, 0.16 mmol) and *N*,*N*′-dimethylethylenediamine (99%, 27 µL, 0.24 mmol), and the reaction mixture was heated in a sealed vial under microwave irradiation (100 °C, 45 min). The mixture was then loaded onto a short SiO_2 plug and washed with 40% EtOAc in hexanes, to provide the crude azide **163** (218 mg, 97%), as a pale yellow oil which was used without further purification.

General procedure B was then used to couple azide **163** (124 mg, 0.55 mmol) and homoprolinol **(*S*)-57** (65 mg, 0.50 mmol). Silica flash column chromatography (0% → 5% MeOH in $CHCl_3$ with 1% NEt_3), furnished the *title compound* (58 mg, 35%) as a yellow oil.

R_f 0.24 (10% MeOH in $CHCl_3$ with 1% NEt_3); ν_{max} (ATR) 3026 (w), 2942 (w), 2870 (w), 2774 (w), 2117 (s), 1504 (s), 1453 (m), 1366 (m), 1285 (s), 1217 (m), 1092 (s) cm^{-1}; δ_H (700 MHz, $CDCl_3$, mixture of diastereomers) 7.34 – 7.30 (6H, m, Ar***H***), 7.26 – 7.23 (1H, m, Ar***H***), 7.00 – 6.95 (2H, m, Ar***H***), 5.30 (1H, s, 1″-***H***), 3.53 – 3.43 (2H, m, 2′-***H***$_2$), 3.05 (1H, ddd, *J* = 9.5, 8.0, 2.0 Hz, 5-***H***H′), 2.31 (3H, apparent d, *J* = 2.0 Hz, NC***H***$_3$ isomer 1, NC***H***$_3$ isomer 2), 2.18 – 2.05 (3H, m, 1′-***H***H′, 2-***H***, 5-H***H***′), 1.93 – 1.86 (1H, m, 3-***H***H′), 1.78 – 1.70 (1H, m, 4-***H***H′), 1.69 – 1.62 (1H, m, 4-H***H***′), 1.57 – 1.50 (1H, m, 1′-H***H***′), 1.49 – 1.41 (1H, m, 3-H***H***′); δ_C (176 MHz, $CDCl_3$, mixture of diastereomers) 142.4 (Ar***C***), 142.3 (Ar***C***), 139.7 (Ar***C***), 139.6 (Ar***C***), 139.1(8) (***C***-4‴ isomer 1), 139.1(5) (***C***-4‴ isomer 2), 128.6 (Ar***C***), 128.5(3) (Ar***C***), 128.4(8) (Ar***C***), 127.6(8) (Ar***C***), 127.6(5) (Ar***C***), 127.0(1) (Ar***C***), 126.9(6) (Ar***C***), 119.1(2) (Ar***C***), 119.1(1) (Ar***C***), 83.3(1) (***C***-1″ isomer 1), 83.3(0) (***C***-1″ isomer 2), 67.2(7) (***C***-2′ isomer 1), 67.2(6) (***C***-2′ isomer 2), 63.9(2) (***C***-2 isomer 1),

63.9(1) (***C***-2 isomer 2), 57.3 (***C***-5), 40.7 (N***C***H_3), 34.2 (***C***-1'), 31.0(9) (***C***-3 isomer 1), 31.0(8) (***C***-3 isomer 2), 22.1 (***C***-4); *m/z* (LCMS, ESI^+) 337 (MH^+); Accurate mass: Found MH^+, 337.2036: $C_{20}H_{25}N_4O$ requires *M*, 337.2028.

4-Bromophenyl(phenyl)methanol[296] 164

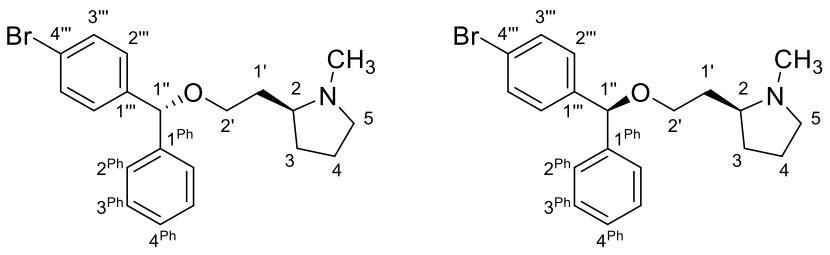


Procedure: 4-Bromobenzaldehyde **165** (2.63 g, 14.23 mmol) was reacted according to general procedure C, furnishing the title compound (3.54 g, 95%) as a white solid after chromatography on silica (0% → 30% ether in hexanes).

R_f 0.33 (20% EtOAc in hexanes); M.p. 61 – 62 °C (lit.:[297] 62 – 63 °C); ν_{max} (ATR) 3352 (br), 3087 (w), 3063 (w), 3028 (w), 2877 (w), 1485 (s), 1455 (m), 1366 (m), 1218 (m), 1071 (m), 1010 (s) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 7.44 (2H, d, *J* = 8.0 Hz, 3-***H***$_2$), 7.35 – 7.31 (4H, m, 2^{Ph}-***H***$_2$, 3^{Ph}-***H***$_2$), 7.30 – 7.26 (1H, m, 4^{Ph}-***H***), 7.26 – 7.22 (2H, m, 2-***H***$_2$), 5.76 (1H, s, C***H***OH), 2.40 (1H, s, CHO***H***); δ_C (176 MHz, $CDCl_3$) 143.5 (***C***-1^{Ph}), 142.9 (***C***-1), 131.7 (***C***-3), 128.8 (***C***-3^{Ph}), 128.3 (***C***-2), 128.0 (***C***-4^{Ph}), 126.7 (***C***-2^{Ph}), 121.5 (***C***-4), 75.8 (***C***HOH); *m/z* (LCMS, ESI^+) 245 (M(^{79}Br) – OH^-), 247 (M(^{81}Br) – OH^-); Accurate mass: Found M – OH^-, 244.9973: $C_{13}H_{10}{}^{79}Br$ requires *M*, 244.9966.

2-(2'-[{4'''-Bromophenyl}{phenyl}methoxy]ethyl)-1-methylpyrrolidine 166

A mixture of (2*S*,1''*R*) and (2*S*,1''*S*) diastereomers

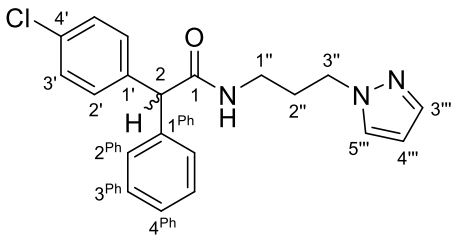


Procedure: General procedure B was used in the reaction of bromide **164** (226 mg, 0.86 mmol) and homoprolinol **(*S*)-57** (101 mg, 0.78 mmol). Reversed-phase column chromatography

(5% → 100% MeCN in H_2O with 0.1% formic acid), furnished the *title compound* (89 mg, 30%) as a clear colourless oil.

R_f 0.29 (10% MeOH in $CHCl_3$ with 1% NEt_3);ν_{max} (ATR) 3033 (w), 3945 (w), 2870 (w), 2775 (w), 1486 (m), 1453 (m), 1366 (s), 1218 (s), 1092 (s), 1010 (s) cm^{-1}; δ_H (700 MHz, $CDCl_3$, mixture of diastereomers) 7.43 (2H, dd, *J* = 8.5, 2.5 Hz, 3‴-***H***$_2$), 7.35 – 7.28 (4H, m, 2^{Ph}-***H***$_2$, 3^{Ph}-***H***$_2$), 7.26 – 7.23 (1H, m, 4^{Ph}-***H***), 7.21 (2H, dd, *J* = 8.5, 2.5 Hz, 2‴-***H***$_2$), 5.27 (1H, s, 1′′-***H***), 3.55 – 3.49 (1H, m, 2′-***H***H′), 3.49 – 3.44 (1H, m, 2′-H***H***′), 3.14 (1H, apparent t, *J* = 9.0 Hz, 5-***H***H′), 2.36 (3H, app d, *J* = 5.0, NC***H***$_3$ isomer 1, NC***H***$_3$ isomer 2), 2.32 – 2.25 (1H, m, 2-***H***), 2.20 (1H, apparent q, *J* = 9.0 Hz, 5-H***H***′), 2.14 – 2.07 (1H, m, 1′-***H***H′), 1.97 – 1.88 (1H, m, 3-***H***H′), 1.84 – 1.74 (1H, m, 4-***H***H′), 1.74 – 1.66 (1H, m, 4-H***H***′), 1.65 – 1.57 (1H, m, 1′-H***H***′), 1.55 – 1.46 (1H, m, 3-H***H***′); δ_C (176 MHz, $CDCl_3$, mixture of diastereomers) 142.0(0) (***C***-1^{Ph} isomer 1), 141.9(6) (***C***-1^{Ph} isomer 2), 141.7(2) (***C***-1‴ isomer 1), 141.7(0) (***C***-1‴ isomer 2), 131.6(0) (***C***-3‴ isomer 1), 131.5(9) (***C***-3‴ isomer 2), 128.7(2) (***C***-2‴ isomer 1), 128.6(8) (***C***-2‴ isomer 2), 128.6 (***C***-3^{Ph}), 127.8(0) (***C***-4^{Ph} isomer 1), 127.7(7) (***C***-4^{Ph} isomer 2), 127.0(3) (***C***-2^{Ph} isomer 1), 126.9(7) (***C***-2^{Ph} isomer 2), 121.4(1) (***C***-4‴ isomer 1), 121.3(8) (***C***-4‴ isomer 2), 83.3(0) (***C***-1′′ isomer 1), 83.2(7) (***C***-1′′ isomer 2), 67.1(3) (***C***-2′ isomer 1), 67.0(9) (***C***-2′ isomer 2), 64.2 (***C***-2), 57.1 (***C***-5), 40.5 (N***C***H_3), 33.8 (***C***-1′), 31.0 (***C***-3 isomer 1), 30.9 (***C***-3 isomer 2), 22.0(4) (***C***-4 isomer 1), 22.0(3) (***C***-4 isomer 2); *m/z* (LCMS, ESI^+) 374 ($M(^{79}Br)H^+$), 376 ($M(^{81}Br)H^+$); Accurate mass: Found MH^+, 374.1134: $C_{20}H_{25}NO^{79}Br$ requires *M*, 374.1120.

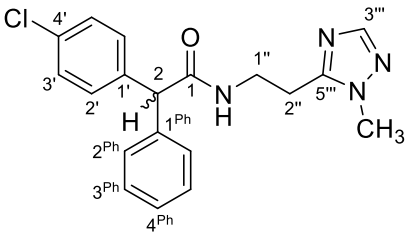
***N*-(3′′-[Pyrazol-1′′′-yl]prop-1′′-yl)-2-(4′-chlorophenyl)-2-phenylacetamide 167**



Procedure: Acid **90** (50 mg, 0.20 mmol) and 3-[pyrazol-1′-yl]propylamine (25 mg, 0.20 mmol) were reacted according to general procedure A. Following reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (31 mg, 44%) was isolated as a white solid.

M.p. 61 – 63 °C; ν_{max} (ATR) 3300 (br), 3072 (w), 3018 (w), 2942 (w), 1646 (s), 1544 (m), 1489 (s), 1402 (m), 1368 (m), 1275 (m), 1212 (m), 1180 (w), 1092 (m), 1019 (s) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 7.43 (1H, dd, *J* = 2.0, 0.5 Hz, 3′′′-***H***), 7.34 – 7.31 (3H, m, Ar***H***), 7.30 – 7.26 (3H, m, Ar***H***), 7.25 – 7.22 (2H, m, Ar***H***), 7.21 – 7.18 (2H, m, Ar***H***), 6.23 – 6.19 (1H, m, 4′′′-***H***), 6.16 (1H, t, *J* = 5.5 Hz, N***H***), 4.78 (1H, s, 2-***H***), 4.13 (2H, t, *J* = 6.5 Hz, 3′′-$\boldsymbol{H_2}$), 3.32 – 3.22 (2H, m, 1′′-$\boldsymbol{H_2}$), 2.02 (2H, p, *J* = 6.5 Hz, 2′′-$\boldsymbol{H_2}$); δ_C (176 MHz, $CDCl_3$) 171.7 (***C***=O), 139.5 (***C***-3′′′), 139.2 (Ar***C***), 138.1 (Ar***C***), 133.3 (***C***-4′), 130.4 (Ar***C***), 129.5 (***C***-5′′′), 129.0 (Ar***C***), 128.9 (Ar***C***), 128.8 (Ar***C***), 127.6 (Ar***C***), 105.9 (***C***-4′′′), 58.6 (***C***-2), 49.7 (***C***-3′′), 37.4 (***C***-1′′), 30.2 (***C***-2′′); *m/z* (LCMS, ESI^+) 354 ($M(^{35}Cl)H^+$), 356 ($M(^{37}Cl)H^+$); Accurate mass: Found MH^+, 354.1378: $C_{20}H_{21}N_3O^{35}Cl$ requires *M*, 354.1373.

***N*-(2′′-[*N*-Methyl-1′′′,2′′′,4′′′-triazol-5′′′-yl]eth-1′′-yl)-2-(4′-chlorophenyl)-2-phenylacetamide 168**

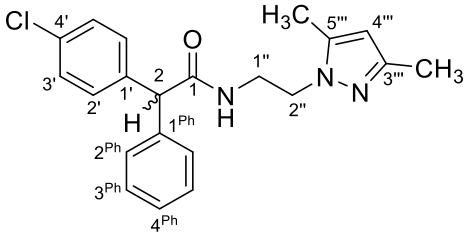


Procedure: Acid **90** (50 mg, 0.20 mmol) and 2-[*N*-methyl-1′,2′,4′-triazol-5′-yl]ethylamine (25 mg, 0.20 mmol) were reacted according to general procedure A. Following reversed-phase

column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (22 mg, 31%) was isolated as a clear colourless oil.

ν_{max} (ATR) 3308 (br), 3040 (w), 2958 (w), 1655 (s), 1524 (w), 1491 (s), 1368 (m), 1222 (m), 1088 (w), 1015 (w) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 7.69 (1H, s, 3‴-***H***), 7.32 – 7.29 (2H, m, 3^{Ph}-***H***$_2$), 7.28 – 7.25 (3H, m, 3′-***H***$_2$, 4^{Ph}-***H***), 7.19 – 7.17 (2H, m, 2^{Ph}-***H***$_2$), 7.16 – 7.14 (2H, m, 2′-***H***$_2$), 6.67 – 6.63 (1H, m, N***H***), 4.80 (1H, s, 2-***H***), 3.75 (3H, s, NC***H***$_3$), 3.74 (2H, q, *J* = 6.0 Hz, 1″-***H***$_2$), 2.91 (2H, t, *J* = 6.0 Hz, 2″-***H***$_2$); δ_C (176 MHz, $CDCl_3$) 171.9 (***C***=O), 153.3 (***C***-5‴), 150.2 (***C***-3‴), 138.9 (***C***-1^{Ph}), 137.8 (***C***-1′), 133.3 (***C***-4′), 130.3 (***C***-2′), 129.0(1) (Ar***C***), 128.9(5) (Ar***C***), 128.8 (***C***-2^{Ph}), 127.6 (***C***-4^{Ph}), 58.6 (***C***-2), 36.9 (***C***-1″), 35.2 (N***C***H_3), 25.5 (***C***-2″); *m/z* (LCMS, ESI^+) 355 ($M(^{35}Cl)H^+$), 357 ($M(^{37}Cl)H^+$); Accurate mass: Found MH^+, 355.1329: $C_{19}H_{20}N_4O^{35}Cl$ requires *M*, 355.1326.

_N_-(2″-[3‴,5‴-Dimethylpyrazol-1‴-yl]eth-1″-yl)-2-(4′-chlorophenyl)-2-phenylacetamide 169

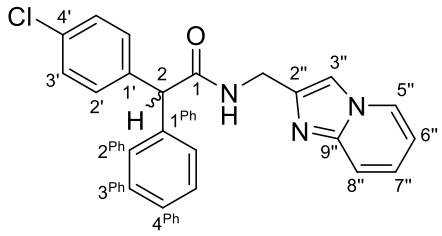


Procedure: Acid **90** (50 mg, 0.20 mmol) and 2-[3′,5′-dimethylpyrazol-1′-yl]ethylamine (28 mg, 0.20 mmol) were reacted according to general procedure A. Following reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (36 mg, 49%) was isolated as a white solid.

M.p. 100 – 101 °C; ν_{max} (ATR) 3282 (br), 3086 (w), 3060 (w), 2922 (w), 1649 (s), 1551 (s), 1489 (s), 1453 (m), 1360 (w), 1222 (w), 1090 (m), 1015 (m) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 7.32 – 7.29 (2H, m, 3^{Ph}-***H***$_2$), 7.28 – 7.24 (3H, m, 3′-***H***$_2$, 4^{Ph}-***H***), 7.20 – 7.18 (2H, m, 2^{Ph}-***H***$_2$), 7.17 – 7.14 (2H, m, 2′-***H***$_2$), 6.49 (1H, t, *J* = 6.0 Hz, N***H***), 5.75 (1H, s, 4‴-***H***), 4.82 (1H, s, 2-***H***), 4.03 (2H, dd, *J* = 6.5, 5.0 Hz, 2″-***H***$_2$), 3.69 (2H, ddd, *J* = 6.5, 6.0, 5.0 Hz, 1″-***H***$_2$), 2.12 (3H, s, 5‴-C***H***$_3$), 2.11 (3H, s, 3‴-C***H***$_3$); δ_C (176 MHz, $CDCl_3$) 171.9 (***C***=O), 148.1 (***C***-3‴), 139.6 (***C***-5‴), 138.9 (***C***-1^{Ph}), 137.9 (***C***-1′), 133.3

(***C***-4′), 130.4 (***C***-2′), 129.0 (Ar***C***), 128.9(4) (Ar***C***), 128.8(8) (Ar***C***), 127.6 (***C***-4^{Ph}), 105.3 (***C***-4‴), 58.7 (***C***-2), 46.9 (***C***-2″), 39.8 (***C***-1″), 13.6 (3‴-***C***H_3), 11.0 (5‴-***C***H_3); *m/z* (LCMS, ESI^+) 368 ($M(^{35}Cl)H^+$), 370 ($M(^{37}Cl)H^+$); Accurate mass: Found MH^+, 368.1525: $C_{21}H_{23}N_3O^{35}Cl$ requires *M*, 368.1530.

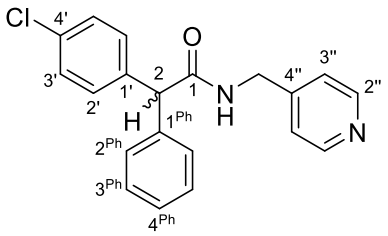
_N_-([Imidazo[1,2-*a*]pyridin-2″-yl]methyl)-2-(4′-chlorophenyl)-2-phenylacetamide 170



Procedure: Acid **90** (50 mg, 0.20 mmol) and (imidazo[1,2-a]pyridin-2-yl)methylamine (29 mg, 0.20 mmol) were reacted according to general procedure A. Following reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (10 mg, 13%) was isolated as a clear yellow oil.

ν_{max} (ATR) 3282 (br), 3062 (w), 3020 (w), 1668 (s), 1534 (w), 1490 (s), 1364 (w), 1208 (m), 1136 (w), 1018 (w) cm^{-1}; δ_H (600 MHz, $CDCl_3$) 8.10 (1H, d, *J* = 7.0 Hz, 5″-***H***), 7.69 – 7.62 (1H, m, N***H***), 7.65 (1H, d, *J* = 9.0 Hz, 8″-***H***), 7.54 (1H, s, 3″-***H***), 7.39 – 7.36 (1H, m, 7″-***H***), 7.25 – 7.23 (4H, m, 2^{Ph}-$\boldsymbol{H}_2$, Ar***H***), 7.23 – 7.19 (5H, m, 2′-$\boldsymbol{H}_2$, Ar***H***), 6.96 (1H, t, *J* = 7.0 Hz, 6″-***H***), 4.89 (1H, s, 2-***H***), 4.60 (2H, t, *J* = 5.5 Hz, NHC$\boldsymbol{H}_2$); δ_C (151 MHz, $CDCl_3$) 172.1 (***C***=O), 143.3 (***C***-9″), 140.4 (***C***-2″), 139.1 (***C***-1^{Ph}), 138.1 (***C***-1′), 133.1 (***C***-4′), 130.4 (Ar***C***), 128.8(4) (Ar***C***), 128.7(7) (Ar***C***), 127.9 (***C***-7″), 127.5 (Ar***C***), 126.4 (***C***-5″), 115.9 (***C***-8″), 114.4 (***C***-6″), 110.9 (***C***-3″), 57.9 (***C***-2), 36.6 (NH***C***H_2); *m/z* (LCMS, ESI^+) 376 ($M(^{35}Cl)H^+$), 378 ($M(^{37}Cl)H^+$); Accurate mass: Found MH^+, 376.1211: $C_{22}H_{19}N_3O^{35}Cl$ requires *M*, 376.1217.

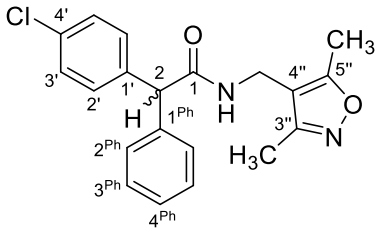
***N*-([Pyridin-4″-yl]methyl)-2-(4′-chlorophenyl)-2-phenylacetamide 171**



Procedure: Acid **90** (50 mg, 0.20 mmol) and (pyridin-4-yl)methylamine (22 mg, 0.20 mmol) were reacted according to general procedure A. Following reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (27 mg, 40%) was isolated as a white solid.

M.p. 148 – 149 °C; ν_{max} (ATR) 3094 (w), 3022 (w), 2946 (w), 1656 (s), 1371 (m), 1218 (m) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 8.55 – 8.52 (2H, m, 2′′-***H***$_2$), 7.37 – 7.34 (2H, m, 3^{Ph}-***H***$_2$), 7.32 – 7.29 (3H, m, 3′-***H***$_2$, 4^{Ph}-***H***$_2$), 7.26 – 7.24 (2H, m, 2^{Ph}-***H***$_2$), 7.23 – 7.20 (2H, m, 2′-***H***$_2$), 7.11 – 7.09 (2H, m, 3′′-***H***$_2$), 6.00 – 5.95 (1H, m, N***H***), 4.94 (1H, s, 2-***H***), 4.49 (2H, d, *J* = 6.0 Hz, NHC***H***$_2$); δ_C (101 MHz, $CDCl_3$) 171.9 (***C***=O), 150.1 (***C***-2′′), 147.4 (***C***-4′′), 138.8 (***C***-1^{Ph}), 137.7 (***C***-1′), 133.5 (***C***-4′), 130.3 (***C***-2′), 129.2 (***C***-3^{Ph}), 129.0 (***C***-3′), 128.8 (***C***-2^{Ph}), 127.9 (***C***-4^{Ph}), 122.3 (***C***-3′′), 58.3 (***C***-2), 42.7 (NH***C***H_2); *m/z* (LCMS, ESI^+) 337 ($M(^{35}Cl)H^+$), 339 ($M(^{37}Cl)H^+$); Accurate mass: Found MH^+, 337.1110: $C_{20}H_{18}N_2O^{35}Cl$ requires *M*, 337.1108.

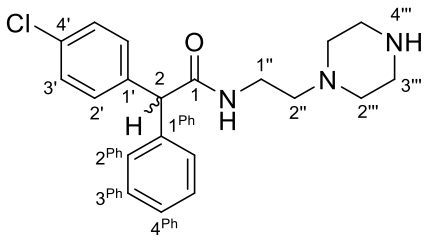
***N*-([3″,5″-Dimethylisoxazol-4″-yl]methyl)-2-(4′-chlorophenyl)-2-phenylacetamide 172**



Procedure: Acid **90** (50 mg, 0.20 mmol) and (3,5-dimethylisoxazol-4-yl]methylamine·HCl (33 mg, 0.20 mmol) were reacted according to general procedure A. Following reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (10 mg, 14%) was isolated as a white solid.

M.p. 169 – 171 °C; ν_{max} (ATR) 3290 (br), 3090 (w), 3044 (w), 2930 (w), 1647 (s), 1548 (m), 1491 (s), 1454 (m), 1200 (w), 1091 (w), 1015 (w) cm^{-1}; δ_H (600 MHz, $CDCl_3$) 7.35 – 7.31 (2H, m, 3^{Ph}-***H***$_2$), 7.31 – 7.27 (3H, m, 3′-***H***$_2$, 4^{Ph}-***H***), 7.20 (2H, d, *J* = 7.5 Hz, 2^{Ph}-***H***$_2$), 7.17 (2H, d, *J* = 8.5 Hz, 2′-***H***$_2$), 5.64 – 5.58 (1H, m, N***H***), 4.83 (1H, s, 2-***H***), 4.20 (2H, d, *J* = 5.5 Hz, NHC***H***$_2$), 2.33 (3H, s, 5′′-C***H***$_3$), 2.11 (3H, s, 3′′-C***H***$_3$); δ_C (151 MHz, $CDCl_3$) 171.4 (***C***=O), 167.3 (***C***-5′′), 159.4 (***C***-3′′), 138.8 (***C***-1^{Ph}), 137.6 (***C***-1′), 133.5 (***C***-4′), 130.3 (***C***-2′), 129.2 (***C***-3^{Ph}), 129.1 (***C***-3′), 128.8 (***C***-2^{Ph}), 127.9 (***C***-4^{Ph}), 110.8 (***C***-4′′), 58.5 (***C***-2), 32.5 (NH***C***H_2), 11.2 (5′′-***C***H_3), 10.2 (3′′-***C***H_3); *m/z* (LCMS, ESI^+) 355 ($M(^{35}Cl)H^+$), 357 ($M(^{37}Cl)H^+$); Accurate mass: Found MH^+, 355.1203: $C_{20}H_{20}N_2O_2{}^{35}Cl$ requires *M*, 355.1213.

_N_-(2′′-[Piperazin-1′′′-yl]eth-1′′-yl)-2-(4′-chlorophenyl)-2-phenylacetamide 173

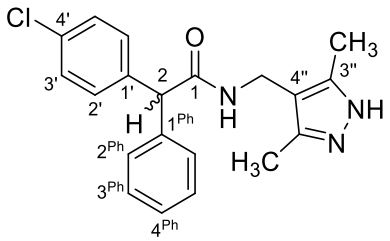


Procedure: Acid **90** (50 mg, 0.20 mmol) and 2-(4′-boc-piperazin-1′-yl)ethylamine (46 mg, 0.20 mmol) were reacted according to general procedure A. Following TFA-mediated deprotection and reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (18 mg, 25%) was isolated as a yellow oil.

ν_{max} (ATR) 3298 (br), 3040 (w), 2942 (w), 2818 (w), 1649 (s), 1556 (m), 1490 (s), 1444 (w), 1320 (w), 1226 (w), 1139 (w), 1089 (w) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 7.35 – 7.31 (2H, m, 3^{Ph}-***H***$_2$), 7.31 – 7.27 (3H, m, 3′-***H***$_2$, 4^{Ph}-***H***), 7.25 – 7.22 (2H, m, 2^{Ph}-***H***$_2$), 7.22 – 7.20 (2H, m, 2′-***H***$_2$), 6.30 – 6.21 (1H, m, N***H***), 4.90 (1H, s, 2-***H***), 3.39 – 3.30 (2H, m, 1′′-***H***$_2$), 2.76 – 2.66 (4H, m, 3′′′-(***H***$_2$)$_2$), 2.40 (2H, app td, *J* = 6.0, 2.0 Hz, 2′′-***H***$_2$), 2.36 – 2.22 (4H, m, 2′′′-(***H***$_2$)$_2$); δ_C (176 MHz, $CDCl_3$) 171.5 (***C***=O), 139.3 (***C***-1_{Ph}), 138.3 (***C***-1′), 133.2 (***C***-4′), 130.5 (***C***-2′), 128.9(9) (***C***-3^{Ph}), 128.9(6) (Ar***C***), 128.9 (Ar***C***), 127.6 (***C***-4^{Ph}), 58.7 (***C***-2), 56.7 (***C***-2′′), 54.2 (***C***-2′′′), 46.2 (***C***-3′′′), 36.1 (***C***-1′′);

m/z (LCMS, ESI$^+$) 358 (M(^{35}Cl)H$^+$), 360 (M(^{37}Cl)H$^+$); Accurate mass: Found MH$^+$, 358.1681: $C_{20}H_{25}N_3O^{35}Cl$ requires *M*, 358.1686.

_N_-([3'',5''-Dimethylpyrazol-4''-yl]methyl)-2-(4'-chlorophenyl)-2-phenylacetamide 174

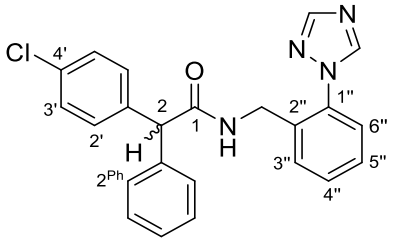


Procedure: Acid **90** (50 mg, 0.20 mmol) and (3,5-dimethylpyrazol-4-yl)methylamine·2HCl (40 mg, 0.20 mmol) were reacted according to general procedure A. Following reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (24 mg, 34%) was isolated as a white solid.

M.p. 177 – 178 °C; ν_{max} (ATR) 3262 (br), 3090 (w), 3032 (w), 2934 (w), 1637 (s), 1540 (m), 1494 (m), 1208 (m), 1090 (w) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 7.34 – 7.31 (2H, m, 3^{Ph}-***H***$_2$), 7.30 – 7.27 (3H, m, 3'-***H***$_2$, 4^{Ph}-***H***), 7.22 – 7.20 (2H, m, 2^{Ph}-***H***$_2$), 7.19 – 7.17 (2H, m, 2'-***H***$_2$), 5.62 – 5.59 (1H, m, N***H***), 4.83 (1H, s, 2-***H***), 4.25 (2H, d, *J* = 5.2 Hz, NHC***H***$_2$), 2.18 (6H, s, 3''-(C***H***$_3$)$_2$); δ_C (176 MHz, $CDCl_3$) 171.3 (***C***=O), 143.4 (***C***-3''), 139.0 (***C***-1^{Ph}), 137.9 (***C***-1'), 133.4 (***C***-4'), 130.3 (***C***-2'), 129.1 (Ar***C***), 129.0 (Ar***C***), 128.8 (Ar***C***), 127.8 (Ar***C***), 112.8 (***C***-4''), 58.5 (***C***-2), 33.2 (NH***C***H$_2$), 10.7 (3''-(***C***H$_3$)$_2$); *m/z* (LCMS, ESI$^+$) 354 (M(^{35}Cl)H$^+$), 356 (M(^{37}Cl)H$^+$); Accurate mass: Found MH$^+$, 354.1384: $C_{20}H_{21}N_3O^{35}Cl$ requires *M*, 354.1373.

***N*-([1''-{1''',2''',4'''-Triazol-1'''-yl}phen-2''-yl]methyl)-2-(4'-chlorophenyl)-2-phenylacetamide**

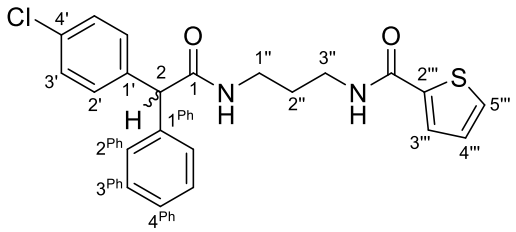
175



Procedure: Acid **90** (50 mg, 0.20 mmol) and 1-([1'',2'',4''-triazol-1''-yl]phen-2'-yl)methylamine (35 mg, 0.20 mmol) were reacted according to general procedure A. Following reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (65 mg, 80%) was isolated as a white solid.

M.p. 137 – 139 °C; ν_{max} (ATR) 3286 (br), 3098 (w), 3044 (w), 2934 (w), 1662 (s), 1494 (s), 1352 (w), 1276 (m), 1215 (m) cm^{-1}; δ_H (700 MHz, $CHCl_3$) 8.30 (1H, s, Ar***H***), 7.90 (1H, s, Ar***H***), 7.66 (1H, dd, *J* = 7.5, 1.5 Hz, 3''-***H***), 7.47 (1H, td, *J* = 7.5, 1.5 Hz, 4''-***H***), 7.43 (1H, td, *J* = 7.5, 1.5 Hz, 5''-***H***), 7.32 – 7.24 (6H, m, Ar***H***), 7.18 – 7.16 (2H, m, 2^{Ph}-$\boldsymbol{H}_2$), 7.14 – 7.12 (2H, m, 2'-$\boldsymbol{H}_2$), 6.94 (1H, t, *J* = 6.5 Hz, N***H***), 4.87 (1H, s, 2-***H***), 4.32 (2H, d, *J* = 6.5 Hz, NHC$\boldsymbol{H}_2$); δ_C (176 MHz, $CDCl_3$) 171.4 (***C***=O), 152.5 (Ar***C***), 143.8 (Ar***C***), 139.1 (Ar***C***), 138.1 (Ar***C***), 135.9 (***C***-2''), 133.6 (***C***-1''), 133.2 (***C***-4'), 132.4 (***C***-3''), 130.4 (***C***-2'), 130.2 (***C***-4''), 129.1 (Ar***C***), 128.9(74) (Ar***C***), 128.9(67) (Ar***C***), 128.9 (Ar***C***), 127.5 (Ar***C***), 124.9 (***C***-6''), 58.6 (***C***-2), 40.5 (NH***C***H_2); *m/z* (LCMS, ESI^+) 403 ($M(^{35}Cl)H^+$), 405 ($M(^{37}Cl)H^+$); Accurate mass: Found MH^+, 403.1327: $C_{23}H_{20}N_4O^{35}Cl$ requires *M*, 403.1326.

***N*-(3''-[Thiophene-2'''-carboxamido]prop-1''-yl)-2-(4'-chlorophenyl)-2-phenylacetamide 176**

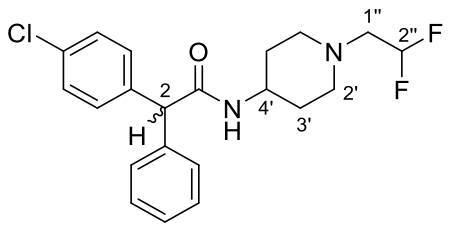


Procedure: Acid **90** (50 mg, 0.20 mmol) and 3-(thiophene-2'-carboxamido)propylamine·HCl (44 mg, 0.20 mmol) were reacted according to general procedure A. Following reversed-phase

column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (45 mg, 54%) was isolated as a white solid.

M.p. 164 – 165 °C; ν_{max} (ATR) 3304 (br), 3076 (w), 2938 (w), 1626 (s), 1543 (s), 1490 (s), 1446 (w), 1353 (w), 1303 (m), 1234 (w), 1090 (m), 1015 (m) cm^{-1}; δ_H (600 MHz, $CHCl_3$) 7.53 (1H, dd, *J* = 3.5, 1.0 Hz, 3‴-***H***), 7.47 – 7.43 (1H, m, 5‴-***H***), 7.34 – 7.31 (2H, m, 3^{Ph}-$\boldsymbol{H}_2$), 7.29 – 7.24 (5H, m, 3′-$\boldsymbol{H}_2$, 2^{Ph}-$\boldsymbol{H}_2$, 4^{Ph}-***H***), 7.23 – 7.21 (2H, m, 2′-$\boldsymbol{H}_2$), 7.13 – 7.07 (1H, m, 3″-N***H***), 7.06 – 7.00 (1H, m, 4‴-***H***), 6.38 – 6.30 (1H, m, 1-N***H***), 4.89 (1H, s, 2-***H***), 3.40 – 3.33 (4H, m, 1″-$\boldsymbol{H}_2$, 3″-$\boldsymbol{H}_2$), 1.70 – 1.63 (2H, m, 2″-$\boldsymbol{H}_2$); δ_C (151 MHz, $CDCl_3$) 172.8 (***C***-1), 162.5 (2‴-***C***O), 139.3 (***C***-2‴), 139.0 (***C***-1^{Ph}), 137.9 (***C***-1′), 133.4 (***C***-4′), 130.3 (***C***-2′), 130.2 (***C***-3^{Ph}), 129.1 (Ar***C***), 129.0 (Ar***C***), 128.8 (***C***-2^{Ph}), 128.1 (***C***-3‴), 127.8 (***C***-4‴), 127.7 (***C***-4^{Ph}), 58.6 (***C***-2), 36.5 (***C***-1″), 36.1 (***C***-3″), 29.9 (***C***-2″); *m/z* (LCMS, ESI^+) 413 ($M(^{35}Cl)H^+$), 415 ($M(^{37}Cl)H^+$); Accurate mass: Found MH^+, 413.1097: $C_{22}H_{22}N_2O_2S^{35}Cl$ requires *M*, 413.1091.

_N_-[1′-(2″,2″-Difluoroethyl)piperidin-4′-yl]-2-(4′-chlorophenyl)-2-phenylacetamide 177

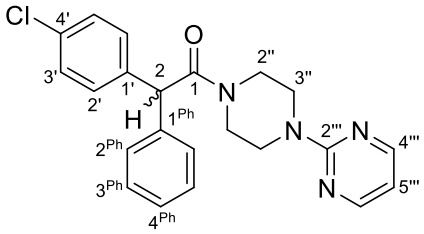


Procedure: Acid **90** (50 mg, 0.20 mmol) and 1-(2′,2′-difluoroethyl)-4-aminopiperidine (33 mg, 0.20 mmol) were reacted according to general procedure A. Following reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (25 mg, 32%) was isolated as an off-white solid.

M.p. 143 – 145 °C; ν_{max} (ATR) 3296 (br), 3058 (w), 2946 (w), 2794 (w), 1639 (s), 1541 (m), 1489 (s), 1290 (w), 1125 (m), 1089 (m), 1049 (s), 1015 (m) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.36 – 7.26 (5H, m, Ar***H***), 7.23 – 7.16 (4H, m, Ar***H***), 5.84 (2H, tt, *J* = 56.0, 4.0 Hz, 2″-***H***), 5.55 – 5.44 (1H, m, N***H***), 4.83 (1H, s, 2-***H***), 3.91 – 3.78 (1H, m, 4′-***H***), 2.90 – 2.79 (2H, m, 2′-$\boldsymbol{H}_2$H′$_2$), 2.72 (2H, td, *J* = 15.0,

4.0 Hz, 1''-***H***$_2$), 2.38 – 2.27 (2H, m, 2'-H$_2$***H***'$_2$), 1.97 – 1.84 (2H, m, 3'-***H***$_2$H'$_2$), 1.48 – 1.32 (2H, m, 3'-H$_2$***H***'$_2$); δ_C (101 MHz, $CDCl_3$) 170.9 (***C***=O), 139.1 (Ar***C***), 138.0 (Ar***C***), 133.3 (Ar***C***), 130.3 (Ar***C***), 129.1 (Ar***C***), 129.0 (Ar***C***), 128.8 (Ar***C***), 127.7 (Ar***C***), 115.5 (t, *J* = 241.5 Hz, ***C***-2''), 59.9 (t, *J* = 25.0 Hz, ***C***-1''), 58.5 (***C***-2), 53.2 (***C***-2'), 46.3 (***C***-4'), 31.9 (***C***-3'); δ_F (376 MHz, $CDCl_3$) −118.52 (dt, *J* = 56.0, 15.0 Hz, 2''-***F***$_2$); *m/z* (LCMS, ESI$^+$) 393 (M(^{35}Cl)H$^+$), 395 (M(^{37}Cl)H$^+$); Accurate mass: Found MH$^+$, 393.1546: $C_{21}H_{24}N_2OF_2{}^{35}Cl$ requires *M*, 393.1545.

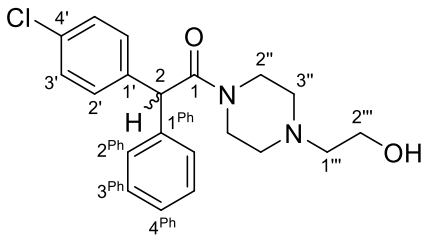
1-(4''-[Pyrimidin-2'''-yl]piperazin-1''-yl)-2-(4'-chlorophenyl)-2-phenylethan-1-one 178



Procedure: Acid **90** (50 mg, 0.20 mmol) and 4-(pyrimidin-2'-yl)piperazine (33 mg, 0.20 mmol) were reacted according to general procedure A. Following reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (18 mg, 23%) was isolated as an off-white solid.

M.p. 150 – 152 °C; ν_{max} (ATR) 3020 (w), 2916 (w), 2856 (w), 1645 (s), 1582 (s), 1548 (s), 1489 (s), 1428 (s), 1392 (m), 1355 (m), 1307 (w), 1261 (m), 1222 (w), 1089 (w), 1032 (w) cm^{-1}; δ_H (600 MHz, $CDCl_3$) 8.28 (2H, d, *J* = 4.5 Hz, 4'''-***H***$_2$), 7.33 (2H, t, *J* = 7.5 Hz, 3^{Ph}-***H***$_2$), 7.29 – 7.22 (5H, m, 3'-***H***$_2$, 2^{Ph}-***H***$_2$, 4^{Ph}-***H***), 7.19 – 7.15 (2H, m, 2'-***H***$_2$), 6.51 (1H, t, *J* = 4.5 Hz, 5'''-***H***), 5.21 (1H, s, 2-***H***), 3.90 – 3.79 (2H, m, 2''-(***H***H')H$_2$, 3''-(***H***H')(HH')), 3.78 – 3.73 (1H, m, 3''-(H***H***')(HH')), 3.70 (1H, ddd, *J* = 13.0, 7.5, 3.0 Hz, 2''-(H***H***')H$_2$), 3.64 (1H, dt, *J* = 13.0, 5.0 Hz, 3''-(HH')(***H***H')), 3.51 (2H, t, *J* = 5.0 Hz, 2''-(HH')***H***$_2$), 3.45 (1H, dt, *J* = 13.0, 5.0 Hz, 3''-(HH')(H***H***')); δ_C (151 MHz, $CDCl_3$) 170.2 (***C***=O), 161.6 (***C***-2'''), 157.9 (***C***-4'''), 138.8 (***C***-1^{Ph}), 138.1 (***C***-1'), 133.2 (***C***-4'), 130.6 (***C***-2'), 129.0 (***C***-3^{Ph}), 128.9 (Ar***C***), 128.8 (Ar***C***), 127.5 (***C***-4^{Ph}), 110.6 (***C***-5'''), 54.5 (***C***-2), 45.9 (***C***-2''), 43.6 (***C***-3''), 42.22 (***C***-2''); *m/z* (LCMS, ESI$^+$) 393 (M(^{35}Cl)H$^+$), 395 (M(^{37}Cl)H$^+$); Accurate mass: Found MH$^+$, 393.1470: $C_{22}H_{22}N_4O^{35}Cl$ requires *M*, 393.1482.

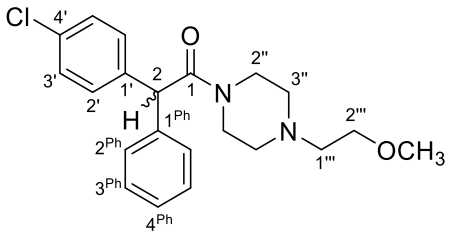
1-(4''-[2'''-Hydroxyethyl]piperazin-1''-yl)-2-(4'-chlorophenyl)-2-phenylethan-1-one 179



Procedure: Acid **90** (50 mg, 0.20 mmol) and 4-(2'-hydroxyethyl)piperazine (27 mg, 0.20 mmol) were reacted according to general procedure A. Following reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (35 mg, 49%) was isolated as a clear yellow oil.

ν_{max} (ATR) 3412 (br), 2938 (w), 2868 (w), 2804 (w), 1638 (s), 1490 (m), 1434 (m), 1290 (w), 1223 (m), 1144 (w), 1087 (m), 1014 (m) cm^{-1}; δ_H (600 MHz, $CDCl_3$) 7.35 – 7.31 (2H, m, 3^{Ph}-***H***$_2$), 7.29 – 7.26 (3H, m, 3'-***H***$_2$, 4^{Ph}-***H***), 7.22 – 7.20 (2H, m, 2^{Ph}-***H***$_2$), 7.16 – 7.13 (2H, m, 2'-***H***$_2$), 5.16 (1H, s, 2-***H***), 3.75 (1H, ddd, *J* = 13.5, 6.5, 3.5 Hz, 2''-(***H***H')H_2), 3.67 (1H, ddd, *J* = 13.5, 6.5, 3.5 Hz, 2''-(H***H***')H_2), 3.59 (2H, t, *J* = 5.5 Hz, 2'''-***H***$_2$), 3.49 – 3.41 (2H, m, 2''-(HH')***H***$_2$), 2.53 – 2.42 (5H, m, 3''-***H***$_2$(HH'), 1'''-***H***$_2$, O***H***), 2.32 – 2.25 (1H, m, 3''-H_2(***H***H')), 2.22 – 2.16 (1H, m, 3''-H_2(H***H***')); δ_C (151 MHz, $CDCl_3$) 169.9 (***C***=O), 138.9 (***C***-1^{Ph}), 138.2 (***C***-1'), 133.1 (***C***-4'), 130.6 (***C***-2'), 129.0 (***C***-3^{Ph}), 128.9 (***C***-2^{Ph}), 128.8 (***C***-3'), 127.5 (***C***-4^{Ph}), 59.3 (***C***-1'''), 57.9 (***C***-2'''), 54.3 (***C***-2), 52.9 (***C***-3''), 52.7 (***C***-3''), 46.1 (***C***-2''), 42.4 (***C***-2''); *m/z* (LCMS, ESI^+) 359 ($M(^{35}Cl)H^+$), 361 ($M(^{37}Cl)H^+$); Accurate mass: Found MH^+, 359.1532: $C_{20}H_{24}N_2O_2{}^{35}Cl$ requires *M*, 359.1526.

1-(4''-[2'''-Methoxyethyl]piperazin-1''-yl)-2-(4'-chlorophenyl)-2-phenylethan-1-one 180

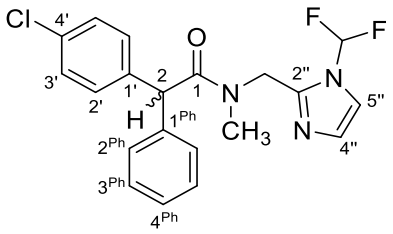


Procedure: Acid **90** (50 mg, 0.20 mmol) and 4-(2'-methoxyethyl)piperazine (29 mg, 0.20 mmol) were reacted according to general procedure A. Following reversed-phase column

chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (8 mg, 11%) was isolated as a clear yellow oil.

ν_{max} (ATR) 2928 (w), 2882 (w), 2808 (w), 1639 (s), 1490 (m), 1432 (m), 1308 (w), 1225 (w), 1113 (m), 1015 (m) cm^{-1}; δ_H (600 MHz, $CDCl_3$) 7.32 – 7.29 (2H, m, 3^{Ph}-***H***$_2$), 7.27 – 7.22 (3H, m, 3′-***H***$_2$, 4^{Ph}-***H***), 7.21 – 7.18 (2H, m, 2^{Ph}-***H***$_2$), 7.14 – 7.11 (2H, m, 2′-***H***$_2$), 5.14 (1H, s, 2-***H***), 3.75 (1H, ddd, *J* = 13.0, 6.0, 3.5 Hz, 2′′-(***H***H′)H_2), 3.66 (1H, ddd, *J* = 13.0, 7.0, 3.5 Hz, 2′′-(H***H***′)H_2), 3.49 – 3.39 (2H, m, 2′′-(HH′)***H***$_2$), 3.45 (2H, t, *J* = 5.5 Hz, 2′′′-***H***$_2$), 3.31 (3H, s, OC***H***$_3$), 2.50 (2H, t, *J* = 5.5 Hz, 1′′′-***H***$_2$), 2.46 (1H, ddd, *J* = 11.0, 6.5, 3.5 Hz, 3′′-(***H***H′)(HH′)), 2.42 (1H, ddd, *J* = 11.0, 7.0, 3.5 Hz, 3′′-(H***H***′)(HH′)), 2.26 (1H, ddd, *J* = 11.0, 6.5, 3.5 Hz, 3′′-(HH′)(***H***H′)), 2.16 (1H, ddd, *J* = 11.0, 6.0, 4.0 Hz, 3′′-(HH′)(H***H***′)); δ_C (151 MHz, $CDCl_3$) 169.8 (***C***=O), 139.0 (***C***-1^{Ph}), 138.3 (***C***-1′), 133.0 (***C***-4′), 130.6 (***C***-2′), 128.8(8) (Ar***C***), 128.8(7) (Ar***C***), 128.7 (***C***-3′), 127.4 (***C***-4^{Ph}), 70.1 (***C***-2′′′), 59.0 (O***C***H_3), 57.9 (***C***-1′′′), 54.2 (***C***-2), 53.5 (***C***-3′′), 53.2 (***C***-3′′), 46.0 (***C***-2′′), 42.2 (***C***-2′′); *m/z* (LCMS, ESI^+) 373 (M(^{35}Cl)H^+), 375 (M(^{37}Cl)H^+); Accurate mass: Found MH^+, 373.1671: $C_{21}H_{26}N_2O_2{}^{35}Cl$ requires *M*, 373.1683.

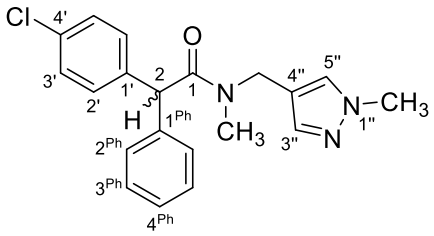
_N_-Methyl-_N_-([1′′-difluoromethylimidazol-2′′-yl]methyl)-2-(4′-chlorophenyl)-2-phenylacetamide 181



Procedure: Acid **90** (50 mg, 0.20 mmol) and *N*-methyl-(1-difluoromethylimidazol-2-yl) methylamine (32 mg, 0.20 mmol) were reacted according to general procedure A. Following reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (37 mg, 47%) was isolated as a clear colourless oil.

ν_{max} (ATR) 3136 (w), 2942 (w), 1639 (s), 1489 (s), 1460 (m), 1416 (m), 1348 (w), 1273 (s), 1192 (w), 1160 (m), 1091 (s), 1047 (s) cm^{-1}; δ_H (600 MHz, $CDCl_3$) 7.77 (1H, t, *J* = 59.0 Hz, C***H***F_2), 7.34 – 7.30 (2H, m, 3^{Ph}-***H***$_2$), 7.29 – 7.26 (4H, m, 3′-***H***$_2$, 4^{Ph}-***H***, 5′′-***H***), 7.18 – 7.14 (2H, m, 2^{Ph}-***H***$_2$), 7.12 – 7.08 (2H, m, 2′-***H***$_2$), 7.04 (1H, d, *J* = 1.5 Hz, 4′′-***H***), 5.17 (1H, s, 2-***H***), 4.78 (1H, d, *J* = 15.0 Hz, N(CH_3)C***H***H′), 4.72 (1H, d, *J* = 15.0 Hz, N(CH_3)CH***H***′), 3.05 (3H, s, NC***H***$_3$CHH′); δ_C (151 MHz, $CDCl_3$) 172.2 (***C***=O), 143.1 (***C***-2′′), 138.1 (***C***-1^{Ph}), 137.4 (***C***-1′), 133.4 (***C***-4′), 130.4 (***C***-2′), 129.7 (***C***-4′′), 129.1 (***C***-3^{Ph}), 128.9 (***C***-3′), 128.7 (***C*** 2^{Ph}), 127.7 (***C***-4^{Ph}), 116.1 (***C***-5′′), 108.4 (t, *J* = 250.0 Hz, ***C***HF_2), 54.3 (***C***-2), 43.4 (N(CH_3)***C***HH′), 35.3 (N***C***H_3CHH′); δ_F (376 MHz, $CDCl_3$) −91.65 (dd, *J* = 231.5, 59.0 Hz, CH***F***F′), −92.72 (dd, *J* = 231.5, 59.0 Hz, CHF***F***′); *m/z* (LCMS, ESI^+) 390 ($M(^{35}Cl)H^+$), 392 ($M(^{37}Cl)H^+$); Accurate mass: Found MH^+, 390.1181: $C_{20}H_{19}N_3OF_2{}^{35}Cl$ requires *M*, 390.1185.

_N_-Methyl-_N_-([1′′-methylpyrazol-4′′-yl]methyl)-2-(4′-chlorophenyl)-2-phenylacetamide 182

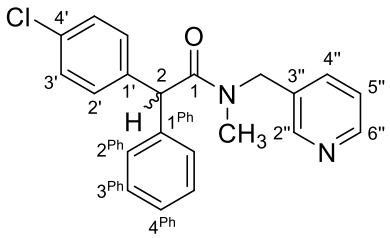


Procedure: Acid **90** (50 mg, 0.20 mmol) and *N*-methyl-(1-methylpyrazol-4-yl)methylamine (32 mg, 0.20 mmol) were reacted according to general procedure A. Following reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (27 mg, 38%) was isolated as a clear colourless oil.

ν_{max} (ATR) 3050 (w), 2932 (w), 2868 (w), 1639 (s), 1489 (s), 1446 (m), 1394 (s), 1334 (w), 1252 (w), 1161 (m), 1088 (m), 1015 (m) cm^{-1}; δ_H (600 MHz, $CDCl_3$, mixture of rotamers) 7.39 (0.7H, s, 3′′-***H*** rotamer A), 7.33 (0.7H, s, 5′′-***H*** rotamer A), 7.32 – 7.28 (2H, m, 3^{Ph}-***H***$_2$), 7.28 – 7.22 (3.6H, m, 3′-***H***$_2$, 2^{Ph}-***H***$_2$ rotamer B, 4^{Ph}-***H***), 7.21 (0.3H, s, 3′′-***H*** rotamer B), 7.22 – 7.17 (1.4H, m, 2^{Ph}-***H***$_2$ rotamer A), 7.16 – 7.14 (0.6H, m, 2′-***H***$_2$ rotamer B), 7.15 – 7.12 (1.4H, m, 2′-***H***$_2$ rotamer A), 6.90 (0.3H, s, 5′′-***H*** rotamer B), 5.25 (0.3H, s, 2-***H*** rotamer B), 5.13 (0.7H, s, 2-***H*** rotamer A), 4.45 (0.7H, d, *J* = 14.5 Hz, N(CH_3)C***H***H′ rotamer A), 4.41 (0.7H, d, *J* = 14.5 Hz, N(CH_3)CH***H***′ rotamer A),

4.37 (0.6H, s, N(CH_3)C***H***$_2$ rotamer B), 3.85 (2.1H, s, 1''-C***H***$_3$ rotamer A), 3.83 (0.9H, s, 1''-C***H***$_3$ rotamer B), 2.98 (0.9H, s, NC***H***$_3$$CH_2$ rotamer B), 2.92 (2.1H, s, NC***H***$_3$CHH' rotamer A); δ_C (151 MHz, $CDCl_3$, mixture of rotamers) 171.2 (***C***=O), 139.3 (***C***-3'' rotamer A), 139.1 (***C***-1^{Ph} rotamer B), 138.9 (***C***-1^{Ph} rotamer A), 138.4 (***C***-1' rotamer B), 138.2 (***C***-1' rotamer A), 138.1 (***C***-3'' rotamer B), 133.1 (***C***-4' rotamer B), 133.0 (***C***-4' rotamer A), 130.5(7) (***C***-2' rotamer B), 130.5(5) (***C***-2' rotamer A), 130.0 (***C***-5'' rotamer A), 129.0 (Ar***C***), 128.9(1) (Ar***C***), 128.8(8) (Ar***C***), 128.8(5) (Ar***C***), 128.7 (Ar***C***), 128.5 (***C***-5'' rotamer B), 127.5 (Ar***C***), 127.4 (Ar***C***), 117.4 (***C***-4'' rotamer B), 117.3 (***C***-4'' rotamer A), 54.4 (***C***-2 rotamer A), 54.1 (***C***-2 rotamer B), 44.8 (N(CH_3)***C***H_2 rotamer B), 42.4 (N(CH_3)***C***HH' rotamer A), 39.2 (1''-***C***H_3 rotamer B), 39.0 (1''-***C***H_3 rotamer A), 35.3 (N(***C***H_3)CHH' rotamer A), 34.1 (N(***C***H_3)CH_2 rotamer B); *m/z* (LCMS, ESI^+) 354 (M(^{35}Cl)H^+), 356 (M(^{37}Cl)H^+); Accurate mass: Found MH^+, 354.1367: $C_{20}H_{21}N_3O^{35}Cl$ requires *M*, 354.1373.

_N_-Methyl-_N_-([pyridin-3''-yl]methyl)-2-(4'-chlorophenyl)-2-phenylacetamide 183

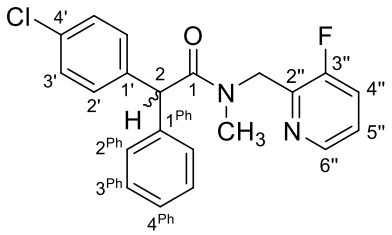


Procedure: Acid **90** (50 mg, 0.20 mmol) and *N*-methyl-(pyridin-3-yl)methylamine (24 mg, 0.20 mmol) were reacted according to general procedure A. Following reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (14 mg, 20%) was isolated as a clear colourless oil.

ν_{max} (ATR) 3036 (w), 2932 (w), 1642 (s), 1489 (m), 1396 (m), 1270 (w), 1174 (w), 1089 (m), 1015 (w) cm^{-1}; δ_H (600 MHz, $CDCl_3$, mixture of rotamers) 8.59 – 8.56 (0.3H, m, 6''-***H*** rotamer B), 8.54 – 8.51 (0.7H, m, 6''-***H*** rotamer A), 8.51 – 8.47 (0.7H, m, 2''-***H*** rotamer A), 8.44 – 8.42 (0.3H, m, 2''-***H*** rotamer B), 7.63 – 7.58 (0.7H, m, 4''-***H*** rotamer A), 7.38 – 7.34 (0.3H, m, 4''-***H*** rotamer B), 7.34 – 7.29 (2H, m, 3^{Ph}-***H***$_2$), 7.29 – 7.24 (4H, m, 3'-***H***$_2$, 4^{Ph}-***H***, 5''-***H***), 7.23 – 7.22 (1.4H, m, 2^{Ph}-***H***$_2$ rotamer A), 7.23 – 7.19 (0.6H, m, 2^{Ph}-***H***$_2$ rotamer B), 7.19 – 7.15 (1.4H, m, 2'-***H***$_2$ rotamer A),

7.14 – 7.10 (0.6H, m, 2′-***H***$_2$ rotamer B), 5.20 (0.7H, s, 2-***H*** rotamer A), 5.11 (0.3H, s, 2-***H*** rotamer B), 4.66 (0.7H, d, *J* = 15.0 Hz, N(CH$_3$)C***H***H′ rotamer A), 4.60 (0.7H, d, *J* = 15.0 Hz, N(CH$_3$)CH***H***′ rotamer A), 4.58 (0.3H, d, *J* = 17.0 Hz, N(CH$_3$)C***H***H′ rotamer B), 4.51 (0.3H, d, *J* = 17.0 Hz, N(CH$_3$)CH***H***′ rotamer B), 3.02 (0.9H, s, N(C***H***$_3$)CHH′ rotamer B), 2.94 (2.1H, s, N(C***H***$_3$)CHH′ rotamer A); δ_C (151 MHz, $CDCl_3$, mixture of rotamers) 171.8(4) (***C***=O rotamer A), 171.8(2) (***C***=O rotamer B), 149.6 (***C***-2′′ rotamer A), 149.5 (***C***-6′′ rotamer B), 149.2 (***C***-6′′ rotamer A), 148.4 (***C***-2′′ rotamer B), 138.7 (***C***-1^{Ph} rotamer B), 138.6 (***C***-1^{Ph} rotamer A), 138.0 (***C***-1′), 136.1 (***C***-4′′ rotamer A), 134.0 (***C***-4′′ rotamer B), 133.3 (***C***-4′ rotamer B), 133.2 (***C***-4′ rotamer A), 132.9 (***C***-3′′ rotamer A), 132.2 (***C***-3′′ rotamer B), 130.5(0) (***C***-2′ rotamer A), 130.4(5) (***C***-2′ rotamer B), 129.1 (Ar***C***), 129.0 (Ar***C***), 128.8(1) (Ar***C***), 128.7(8) (Ar***C***), 127.7 (Ar***C***), 127.5 (Ar***C***), 123.9 (***C***-5′′ rotamer B), 123.8 (***C***-5′′ rotamer A), 54.4 (***C***-2 rotamer A), 54.3 (***C***-2 rotamer B), 51.3 (N(CH$_3$)***C***HH′ rotamer B), 49.4 (N(CH$_3$)***C***HH′ rotamer A), 35.5 (N(***C***H$_3$)HH′ rotamer A), 34.8 (N(***C***H$_3$)HH′ rotamer B); *m/z* (LCMS, ESI$^+$) 351 (M(^{35}Cl)H$^+$), 353 (M(^{37}Cl)H$^+$); Accurate mass: Found MH$^+$, 351.1259: $C_{21}H_{20}N_2O^{35}Cl$ requires *M*, 351.1264.

_N_-Methyl-_N_-([3′′-fluoropyridin-2′′-yl]methyl)-2-(4′-chlorophenyl)-2-phenylacetamide 184

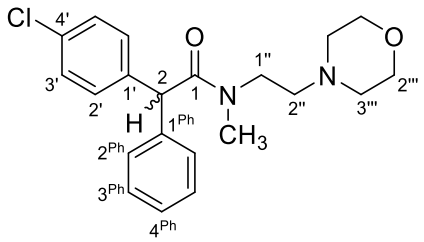


Procedure: Acid **90** (50 mg, 0.20 mmol) and *N*-methyl-(3-fluoropyridin-2-yl)methylamine (28 mg, 0.20 mmol) were reacted according to general procedure A. Following reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (32 mg, 43%) was isolated as a clear colourless oil.

ν_{max} (ATR) 3058 (w), 2928 (w), 1647 (s), 1489 (m), 1443 (s), 1396 (m), 1243 (w), 1159 (w), 1090 (m), 1015 (w) cm^{-1}; δ_H (600 MHz, $CDCl_3$, mixture of rotamers) 8.45 (0.5H, dt, *J* = 5.0, 1.5 Hz, 6′′-***H*** rotamer A), 8.34 (0.5H, dt, *J* = 5.0, 1.5 Hz, 6′′-***H*** rotamer B), 7.40 (0.5H, ddd, *J* = 9.5, 8.5,

1.5 Hz, 4''-***H*** rotamer A), 7.34 (0.5H, ddd, *J* = 9.5, 8.5, 1.5 Hz, 4''-***H*** rotamer B), 7.32 – 7.28 (2.5H, m, 5''-***H*** rotamer A, 3^{Ph}-$\boldsymbol{H}_2$), 7.27 – 7.22 (5H, m, 3'-$\boldsymbol{H}_2$, 2^{Ph}-$\boldsymbol{H}_2$, 4^{Ph}-***H***), 7.22 – 7.18 (1.5H, m, 2'-$\boldsymbol{H}_2$ rotamer B, 5''-***H*** rotamer B), 7.18 – 7.16 (1H, m, 2'-$\boldsymbol{H}_2$ rotamer A), 5.60 (0.5H, s, 2-***H*** rotamer A), 5.27 (0.5H, s, 2-***H*** rotamer B), 4.89 (0.5H, dd, *J* = 15.5, 2.0 Hz, N(CH_3)C***H***H' rotamer B), 4.79 (0.5H, dd, *J* = 15.5, 1.5 Hz, N(CH_3)CH***H***' rotamer B), 4.70 (0.5H, dd, *J* = 16.5, 1.5 Hz, N(CH_3)C***H***H' rotamer A), 4.54 (0.5H, dd, *J* = 16.5, 1.5 Hz, N(CH_3)CH***H***' rotamer A), 3.08 (1.5H, s, N(C$\boldsymbol{H}_3$)CHH' rotamer B), 3.04 (1.5H, s, N(C$\boldsymbol{H}_3$)CHH' rotamer A); δ_C (151 MHz, $CDCl_3$, mixture of rotamers) 172.6 (***C***=O rotamer A), 171.8 (***C***=O rotamer B), 157.9 (d, *J* = 258.5 Hz, ***C***-3'' rotamer B), 157.7 (d, *J* = 257.5 Hz, ***C***-3'' rotamer A), 145.7 (d, *J* = 5.5 Hz, ***C***-6'' rotamer A), 145.2 (d, *J* = 14.5 Hz, ***C***-2'' rotamer B), 145.0 (d, *J* = 5.5 Hz, ***C***-6'' rotamer B), 144.5 (d, *J* = 14.5 Hz, ***C***-2'' rotamer A), 139.2 (***C***-1^{Ph} rotamer A), 139.1 (***C***-1^{Ph} rotamer B), 138.7 (***C***-1' rotamer A), 138.4 (***C***-1' rotamer B), 132.9(3) (***C***-4' rotamer B), 132.8(6) (***C***-4' rotamer A), 130.8 (***C***-2' rotamer B), 130.7 (***C***-2' rotamer A), 129.2 (Ar***C***), 129.0 (Ar***C***), 128.8 (***C***-3^{Ph}), 128.7 (***C***-3^{Ph}), 128.6(1) (Ar***C***), 128.5(8) (Ar***C***), 127.3 (Ar***C***), 124.6 (d, *J* = 3.5 Hz, ***C***-5'' rotamer A), 123.8 (d, *J* = 3.5 Hz, ***C***-5'' rotamer B), 123.3 (d, *J* = 19.0 Hz, ***C***-4'' rotamer A), 122.9 (d, *J* = 18.5 Hz, ***C***-4'' rotamer B), 54.4 (***C***-2 rotamer B), 53.8 (***C***-2 rotamer A), 49.4 (d, *J* = 1.5 Hz, N(CH_3)***C***HH' rotamer A), 48.1 (d, *J* = 1.0 Hz, N(CH_3)***C***HH' rotamer B), 36.8 (N(***C***H_3)CHH' rotamer B), 35.2 (N(***C***H_3)CHH' rotamer A); δ_F (376 MHz, $CDCl_3$) −125.79 (3''-***F***); *m/z* (LCMS, ESI^+) 369 (M(^{35}Cl)H^+), 371 (M(^{37}Cl)H^+); Accurate mass: Found MH^+, 369.1154: $C_{21}H_{19}N_2OF^{35}Cl$ requires *M*, 369.1170.

_N_-Methyl-_N_-(2''-[morpholin-4'''-yl]eth-1''-yl)-2-(4'-chlorophenyl)-2-phenylacetamide 185



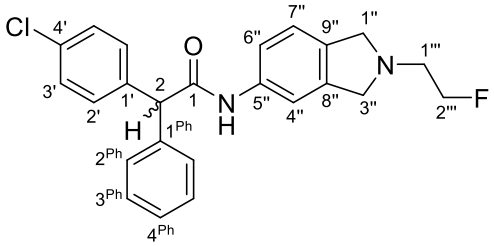
Procedure: Acid **90** (50 mg, 0.20 mmol) and *N*-methyl-2-(morpholin-4'-yl)ethylamine (30 mg, 0.20 mmol) were reacted according to general procedure A. Following reversed-phase column

chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (9 mg, 12%) was isolated as a yellow oil.

ν_{max} (ATR) 2958 (w), 2855 (w), 2812 (w), 1643 (s), 1489 (m), 1456 (w), 1399 (m), 1304 (w), 1260 (w), 1152 (w), 1116 (s), 1015 (w) cm^{-1}; δ_H (600 MHz, $CDCl_3$, mixture of rotamers) 7.34 – 7.30 (2H, m, 3^{Ph}-***H***$_2$), 7.28 – 7.23 (5H, m, 3′-***H***$_2$, 2^{Ph}-***H***$_2$, 4^{Ph}-***H***), 7.19 – 7.15 (2H, m, 2′-***H***$_2$), 5.28 (0.3H, s, 2-***H*** rotamer B), 5.18 (0.7H, s, 2-***H*** rotamer A), 3.71 – 3.64 (4H, m, 2‴-(***H***$_2$)$_2$), 3.60 (0.7H, dt, *J* = 13.5, 6.5 Hz, 1″-***H***H′ rotamer A), 3.53 (0.7H, dt, *J* = 13.5, 6.5 Hz, 1″-H***H***′ rotamer A), 3.43 (0.3H, ddd, *J* = 14.5, 7.5, 6.0 Hz, 1″-***H***H′ rotamer B), 3.36 (0.3H, ddd, *J* = 14.5, 7.5, 6.0 Hz, 1″-H***H***′ rotamer B), 3.01 (0.9H, s, NC***H***$_3$ rotamer B), 2.99 (2.1H, s, NC***H***$_3$ rotamer A), 2.51 (1.4H, t, *J* = 6.5 Hz, 2″-***H***$_2$ rotamer A), 2.52 – 2.45 (2.8H, m, 3‴-(***H***$_2$)$_2$ rotamer A), 2.45 (0.3H, ddd, *J* = 13.0, 7.5, 6.0 Hz, 2″-***H***H′ rotamer B), 2.42 – 2.39 (1.2H, m, 3‴-(***H***$_2$)$_2$ rotamer B), 2.37 (0.3H, ddd, *J* = 13.0, 7.5, 6.0 Hz, 2″-H***H***′ rotamer B); δ_C (151 MHz, $CDCl_3$, mixture of rotamers) 171.5 (***C***=O rotamer B), 171.4 (***C***=O rotamer A), 139.2 (***C***-1^{Ph} rotamer B), 139.0 (***C***-1^{Ph} rotamer A), 138.6 (***C***-1′ rotamer B), 138.4 (***C***-1′ rotamer A), 133.0(4) (***C***-4′ rotamer B), 132.9(7) (***C***-4′ rotamer A), 130.7 (***C***-2′ rotamer A), 130.5 (***C***-2′ rotamer B), 129.0 (Ar***C***), 128.9 (Ar***C***), 128.8(4) (Ar***C***), 128.8(0) (Ar***C***), 128.7(0) (Ar***C***), 128.6(6) (Ar***C***), 127.4 (Ar***C***), 127.3 (Ar***C***), 67.2 (***C***-2‴ rotamer A), 67.0 (***C***-2‴ rotamer B), 57.3 (***C***-2″ rotamer B), 55.8 (***C***-2″ rotamer A), 54.5 (***C***-2 rotamer A), 54.2 (***C***-3‴ rotamer B), 53.8(9) (***C***-3‴ rotamer A), 53.8(6) (***C***-2 rotamer B), 47.6 (***C***-1″ rotamer B), 45.3 (***C***-1″ rotamer A), 36.2 (N***C***H_3 rotamer A), 34.6 (N***C***H_3 rotamer B); *m/z* (LCMS, ESI^+) 373 (M(^{35}Cl)H^+), 375 (M(^{37}Cl)H^+); Accurate mass: Found MH^+, 373.1673: $C_{21}H_{26}N_2O_2{}^{35}Cl$ requires *M*, 373.1683.

***N*-(1′′,3′′-Dihydro-2′′-[2′′′-fluoroethyl]isoindol-5′′-yl)-2-(4′-chlorophenyl)-2-phenylacetamide**

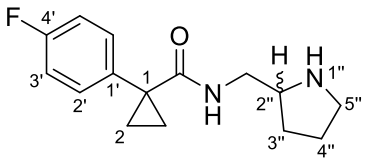
186



Procedure: Acid **90** (50 mg, 0.20 mmol) and 1,3-dihydro-2-[2′-fluoroethyl]-5-aminoisoindole (36 mg, 0.20 mmol) were reacted according to general procedure A. Following reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (7 mg, 9%) was isolated as a brown oil.

ν_{max} (ATR) 3274 (br), 3054 (w), 2946 (w), 2808 (w), 1661 (s), 1602 (w), 1550 (m), 1491 (s), 1442 (w), 1364 (w), 1210 (w), 1096 (w), 1032 (w) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 7.48 (1H, d, *J* = 2.0 Hz, 4′′-***H***), 7.38 – 7.35 (2H, m, 3^{Ph}-$\boldsymbol{H}_2$), 7.33 – 7.31 (3H, m, 3′-$\boldsymbol{H}_2$, 4^{Ph}-***H***), 7.31 – 7.29 (2H, m, 2^{Ph}-$\boldsymbol{H}_2$), 7.26 – 7.24 (2H, m, 2′-$\boldsymbol{H}_2$), 7.13 (1H, dd, *J* = 8.0, 2.0 Hz, 6′′-***H***), 7.10 (1H, d, *J* = 8.0 Hz, 7′′-***H***), 5.01 (1H, s, 2-***H***), 4.62 (2H, dt, *J* = 47.5, 5.0 Hz, 2′′′-$\boldsymbol{H}_2$), 3.97 (4H, s, 3′′-$\boldsymbol{H}_2$, 1′′-$\boldsymbol{H}_2$), 3.04 (2H, dt, *J* = 28.0, 5.0 Hz, 1′′′-$\boldsymbol{H}_2$); δ_C (176 MHz, $CDCl_3$) 169.7 (***C***=O), 141.1 (***C***-5′′), 138.8 (***C***-1^{Ph}), 137.7 (***C***-1′), 136.5(0) (Ar***C***), 136.4(9) (Ar***C***), 133.6 (***C***-4′), 130.5 (***C***-2′), 129.3 (***C***-3^{Ph}), 129.1 (***C***-3′), 129.0 (***C***-2^{Ph}), 127.9 (***C***-4^{Ph}), 122.7 (***C***-7′′), 118.7 (***C***-6′′), 114.4 (***C***-4′′), 83.4 (d, *J* = 167.5 Hz, ***C***-2′′′), 59.7 (***C***-3′′), 59.4 (***C***-2), 59.3 (***C***-1′′), 55.6 (d, *J* = 19.5 Hz, ***C***-1′′′); δ_F (376 MHz, $CDCl_3$) −220.22 – −220.69 (m, 2′′′-***F***); *m/z* (LCMS, ESI^+) 409 ($M(^{35}Cl)H^+$), 411 ($M(^{37}Cl)H^+$); Accurate mass: Found MH^+, 409.1476: $C_{24}H_{23}N_2OF^{35}Cl$ requires *M*, 409.1483.

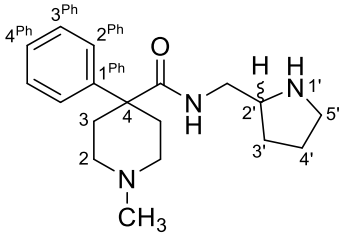
***N*-([Pyrrolidin-2′′-yl]methyl)-1-(4′-fluorophenyl)cyclopropane-1-carboxamide 187**



Procedure: 1-(4′-Fluorophenyl)cyclopropane-1-carboxilic acid (36 mg, 0.20 mmol) and 2-(aminomethyl)-1-Boc-pyrrolidine **91** (40 mg, 0.20 mmol) were reacted according to general procedure A. Following TFA-mediated deprotection and reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (26 mg, 50%) was isolated as a yellow gum.

ν_{max} (ATR) 3326 (br), 2958 (w), 2832 (w), 1658 (m), 1511 (s), 1408 (m), 1214 (m) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 7.37 (2H, dd, *J* = 8.5, 5.5 Hz, 2′-***H***$_2$), 7.04 (2H, t, *J* = 8.5 Hz, 3′-***H***$_2$), 5.76 – 5.70 (1H, m, N***H***), 3.24 (1H, ddd, *J* = 13.0, 6.0, 4.5 Hz, NHC***H***H′), 3.16 (1H, qd, *J* = 7.0, 4.5 Hz, 2′′-***H***), 3.00 (1H, ddd, *J* = 13.0, 7.0, 5.5 Hz, NHCH***H***′), 2.81 (1H, ddd, *J* = 10.5, 7.5, 6.0 Hz, 5′′-***H***H′), 2.73 (1H, dt, *J* = 10.5, 6.5 Hz, 5′′-H***H***′), 1.81 – 1.73 (1H, m, 1′′-***H***), 1.80 – 1.72 (1H, m, 3′′-***H***H′), 1.68 – 1.60 (2H, m, 4′′-***H***$_2$), 1.61 – 1.56 (2H, m, 2-***H***$_2$H′$_2$), 1.26 (1H, ddt, *J* = 13.0, 8.5, 7.0 Hz, 3′′-H***H***′), 1.02 – 0.98 (2H, m, 2-H$_2$***H***′$_2$); δ_C (176 MHz, $CDCl_3$) 173.9 (***C***=O), 162.3 (d, *J* = 247.5 Hz, ***C***-4′), 135.9 (d, *J* = 3.5 Hz, ***C***-1′), 132.8 (d, *J* = 8.0 Hz, ***C***-2′), 116.0 (d, *J* = 21.5 Hz, ***C***-3′), 57.4 (***C***-2′′), 46.7 (***C***-5′′), 44.8 (NH***C***HH′), 29.9 (***C***-1), 29.1 (***C***-3′′), 26.0 (***C***-4′′), 15.8 (***C***-2), 15.7 (***C***-2); δ_F (376 MHz, $CDCl_3$) −114.22 – −114.31 (m, 4′-***F***); *m/z* (LCMS, ESI^+) 263 (MH^+); Accurate mass: Found MH^+, 263.1566: $C_{15}H_{20}N_2OF$ requires *M*, 263.1560.

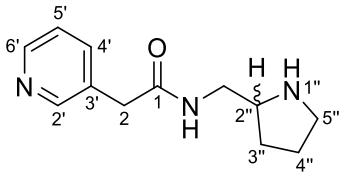
***N*-([Pyrrolidin-2′-yl]methyl)-1-methyl-4-phenyl-piperidine-4-carboxamide 188**



Procedure: 1-Methyl-4-phenylpiperidine-4-carboxilic acid monohydrate (47 mg, 0.20 mmol) and 2-(aminomethyl)-1-Boc-pyrrolidine **91** (40 mg, 0.20 mmol) were reacted according to general procedure A. Following TFA-mediated deprotection and reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (30 mg, 50%) was isolated as a yellow oil.

ν_{max} (ATR) 3308 (br), 2946 (m), 2798 (w), 1640 (s), 1532 (s), 1450 (m), 1412 (w), 1290 (m) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 7.40 – 7.37 (2H, m, 2^{Ph}-***H***$_2$), 7.34 (2H, t, *J* = 7.5 Hz, 3^{Ph}-***H***$_2$), 7.27 – 7.22 (1H, m, 4^{Ph}-***H***), 5.95 – 5.88 (1H, m, N***H***), 3.28 (1H, ddd, *J* = 13.0, 5.0, 4.5 Hz, NHC***H***H′), 3.14 (1H, qd, *J* = 7.0, 4.5 Hz, 2′-***H***), 2.99 (1H, ddd, *J* = 13.0, 7.0, 5.5 Hz, NHCH***H***′), 2.79 (1H, dt, *J* = 10.5, 7.0 Hz, 5′-***H***H′), 2.69 (1H, dt, *J* = 10.5, 6.5 Hz, 5′-H***H***′), 2.66 – 2.52 (2H, m, 2-***H***$_2$H′$_2$), 2.47 – 2.43 (2H, m, 3-***H***$_2$H′$_2$), 2.43 – 2.35 (2H, m, 2-H$_2$***H***′$_2$), 2.25 (3H, s, NC***H***$_3$), 2.14 – 2.06 (2H, m, 3-H$_2$***H***′$_2$), 1.71 (1H, dq, *J* = 12.5, 7.0 Hz, 3′-***H***H′), 1.57 (2H, dtd, *J* = 7.5, 7.0, 6.5 Hz, 4′-***H***$_2$), 1.17 (1H, dtd, *J* = 12.5, 7.5, 7.0 Hz, 3′-H***H***′); δ_C (176 MHz, $CDCl_3$) 174.9 (***C***=O), 143.6 (***C***-1^{Ph}) , 128.9 (***C***-3^{Ph}), 127.0 (***C***-4^{Ph}), 126.2 (***C***-2^{Ph}), 57.4 (***C***-2′), 53.0 (***C***-2), 48.7 (***C***-4), 46.7 (***C***-5′), 46.4 (N***C***H$_3$), 43.9 (NH***C***HH′), 34.2 (***C***-3), 28.9 (***C***-3′), 26.0 (***C***-4′); *m/z* (LCMS, ESI^+) 302 (MH^+); Accurate mass: Found MH^+, 302.2239: $C_{18}H_{28}N_3O$ requires *M*, 302.2232.

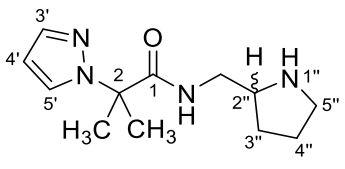
***N*-([Pyrrolidin-2′′-yl]methyl)-2-(pyridin-3′-yl)acetamide[298] 189**



Procedure: 2-(Pyridin-3′-yl)acetic acid·HCl (35 mg, 0.20 mmol) and 2-(aminomethyl)-1-Boc-pyrrolidine **91** (40 mg, 0.20 mmol) were reacted according to general procedure A. Following TFA-mediated deprotection and reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the title compound (13 mg, 30%) was isolated as a yellow oil.

ν_{max} (ATR) 3262 (br), 3034 (w), 2950 (w), 2870 (w), 1650 (s), 1558 (s), 1524 (s), 1478 (w), 1432 (s), 1406 (s), 1360 (m), 1192 (w) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 8.51 – 8.48 (2H, m, 2′-***H***, 6′-***H***), 7.65 (1H, dt, *J* = 8.0, 2.0 Hz, 4′-***H***), 7.24 (1H, dd, *J* = 8.0, 5.0 Hz, 5′-***H***), 6.63 – 6.54 (1H, m, N***H***), 3.51 (2H, s, 2-$\boldsymbol{H}_2$), 3.40 (1H, ddd, *J* = 13.5, 6.0, 4.0 Hz, NHC***H***H′), 3.29 – 3.22 (1H, m, 2′′-***H***), 3.03 (1H, ddd, *J* = 13.5, 8.0, 5.0 Hz, NHCH***H***′), 3.06 – 2.95 (1H, m, 1′′-***H***), 2.89 (1H, ddd, *J* = 10.5, 7.5, 6.0 Hz, 5′′-***H***H′), 2.84 (1H, dt, *J* = 10.5, 7.0 Hz, 5′′-H***H***′), 1.84 (1H, dtd, *J* = 13.0, 8.0, 5.5 Hz, 3′′-***H***H′), 1.77 – 1.70 (1H, m, 4′′-***H***H′), 1.70 – 1.62 (1H, m, 4′′-H***H***′), 1.33 (1H, ddt, *J* = 13.0, 8.5, 7.0 Hz, 3′′-H***H***′); δ_C (176 MHz, $CDCl_3$) 170.1 (***C***=O), 150.4 (***C***-2′), 148.6 (***C***-6′), 136.9 (***C***-4′), 131.1 (***C***-3′), 123.7 (***C***-5′), 57.7 (***C***-2′′), 46.5 (***C***-5′′), 43.7 (NH***C***HH′), 40.8 (***C***-2), 29.2 (***C***-3′′), 25.9 (***C***-4′′); *m/z* (LCMS, ESI^+) 220 (MH^+); Accurate mass: Found MH^+, 220.1453: $C_{12}H_{18}N_3O$ requires *M*, 220.1450.

***N*-([Pyrrolidin-2′′-yl]methyl)-2-methyl-2-(pyrazol-1′-yl)propionamide 190**

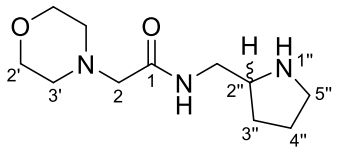


Procedure: 2-Methyl-2-(pyrazol-1′-yl)propionic acid (31 mg, 0.20 mmol) and 2-(aminomethyl)-1-Boc-pyrrolidine **91** (40 mg, 0.20 mmol) were reacted according to general procedure A. Following TFA-mediated deprotection and reversed-phase column chromatography

(5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (21 mg, 45%) was isolated as a yellow oil.

ν_{max} (ATR) 3326 (br), 2938 (w), 2868 (w), 1655 (s), 1516 (br, s), 1394 (s), 1316 (w), 1250 (w), 1200 (w), 1156 (w), 1088 (w), 1058 (w) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 7.63 (1H, d, *J* = 2.0 Hz, Ar***H***), 7.60 (1H, d, *J* = 2.0 Hz, Ar***H***), 6.46 – 6.41 (1H, m, N***H***), 6.32 (1H, t, *J* = 2.0 Hz, 4′-***H***), 3.23 (1H, ddd, *J* = 13.0, 6.0, 4.5 Hz, NHC***H***H′), 3.15 (1H, qd, *J* = 7.0, 4.5 Hz, 2′′-***H***), 3.01 (1H, ddd, *J* = 13.0, 7.0, 5.5 Hz, NHCH***H***′), 2.85 – 2.78 (2H, m, 5′′-$\boldsymbol{H}_2$), 1.85 (3H, s, 2-(C$\boldsymbol{H}_3$)(CH_3)), 1.84 (3H, s, 2-(CH_3)(C$\boldsymbol{H}_3$)), 1.74 (1H, dddd, *J* = 12.5, 8.5, 7.5, 5.5 Hz, 3′′-***H***H′), 1.70 – 1.58 (2H, m, 4′′-$\boldsymbol{H}_2$), 1.23 (1H, ddt, *J* = 12.5, 8.5, 7.0 Hz, 3′′-H***H***′); δ_C (176 MHz, $CDCl_3$) 173.4 (***C***=O), 140.4 (Ar***C***), 128.0 (Ar***C***), 106.2 (***C***-4′), 65.2 (***C***-2), 57.3 (***C***-2′′), 46.6 (***C***-5′′), 44.3 (NH***C***HH′), 29.0 (***C***-3′′), 26.1 (2-(***C***H_3)(CH_3)), 26.02 (2-(CH_3)(***C***H_3)), 25.80 (***C***-4′′); *m/z* (LCMS, ESI^+) 237 (MH^+); Accurate mass: Found MH^+, 237.1723: $C_{12}H_{21}N_4O$ requires *M*, 237.1715.

_N_-([Pyrrolidin-2′′-yl]methyl)-2-(morpholin-4′-yl)acetamide 191

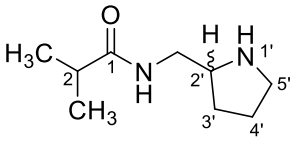


Procedure: 2-(Morpholin-4′-yl)acetic acid·HCl (36 mg, 0.20 mmol) and 2-(aminomethyl)-1-Boc-pyrrolidine **91** (40 mg, 0.20 mmol) were reacted according to general procedure A. Following TFA-mediated deprotection and reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (25 mg, 56%) was isolated as an orange oil.

ν_{max} (ATR) 3340 (br), 2954 (w), 2864 (w), 2812 (w), 1654 (s), 1523 (s), 1404 (m), 1290 (w), 1114 (s), 1014 (w) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 7.47 – 7.39 (1H, m, N***H***), 3.75 – 3.67 (4H, m, 2′-($\boldsymbol{H}_2$)$_2$), 3.40 – 3.33 (1H, m, NHC***H***H′), 3.29 – 3.23 (1H, m, 2′′-***H***), 3.13 – 3.07 (1H, m, NHCH***H***′), 3.02 – 2.97 (2H, m, 2-$\boldsymbol{H}_2$), 2.93 – 2.87 (2H, m, 5′′-$\boldsymbol{H}_2$), 2.56 – 2.47 (4H, m, 3′-($\boldsymbol{H}_2$)$_2$), 1.88 – 1.81 (1H, m, 3′′-***H***H′), 1.78 – 1.72 (1H, m, 4′′-***H***H′), 1.71 – 1.64 (1H, m, 4′′-H***H***′), 1.38 – 1.31 (1H, m, 3′′-H***H***′); δ_C (176 MHz, $CDCl_3$) 170.07 (***C***=O), 67.1 (***C***-2′), 62.2 (***C***-2), 57.9 (***C***-2′′), 54.0 (***C***-3′), 46.8 (***C***-5′′),

43.7 (NH***C***HH'), 29.3 (***C***-3''), 26.0 (***C***-4''); *m/z* (LCMS, ESI^+) 228 (MH^+); Accurate mass: Found MH^+, 228.1706: $C_{11}H_{22}N_3O_2$ requires *M*, 228.1712.

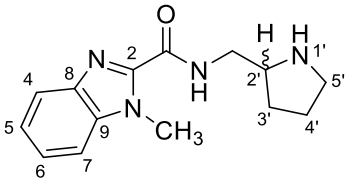
_N_-([Pyrrolidin-2'-yl]methyl)-2-methylpropionamide[299] 192



Procedure: 2-Methylpropionic acid (18 mg, 0.20 mmol) and 2-(aminomethyl)-1-Boc-pyrrolidine **91** (40 mg, 0.20 mmol) were reacted according to general procedure A. Following TFA-mediated deprotection and reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the title compound (20 mg, 59%) was isolated as a yellow oil.

ν_{max} (ATR) 3300 (br), 3058 (w), 2958 (w), 2928 (w), 2872 (w), 1649 (s), 1542 (br, s), 1460 (w), 1394 (s), 1244 (w), 1170 (w), 1110 (w) cm^{-1}; δ_H (600 MHz, $CDCl_3$) 6.08 – 5.98 (1H, m, N***H***), 3.38 (1H, ddd, *J* = 13.0, 6.0, 4.5 Hz, NHC***H***H'), 3.29 – 3.21 (1H, m, 2'-***H***), 3.02 (1H, ddd, *J* = 13.0, 8.0, 5.0 Hz, NHCH***H***'), 2.94 – 2.85 (2H, m, 5'-$\boldsymbol{H}_2$), 2.35 (1H, hept, *J* = 7.0 Hz, 2-***H***), 1.89 – 1.80 (1H, m, 3'-***H***H'), 1.80 – 1.72 (1H, m, 4'-***H***H'), 1.72 – 1.64 (1H, m, 4'-H***H***'), 1.41 – 1.31 (1H, m, 3'-H***H***'), 1.14 (6H, d, *J* = 7.0 Hz, 2-(C$\boldsymbol{H}_3$)(C$\boldsymbol{H}_3$)); δ_C (151 MHz, $CDCl_3$) 177.2 (***C***=O), 57.9 (***C***-2'), 46.7 (***C***-5'), 43.6 (NH***C***HH'), 35.8 (***C***-2), 29.2 (***C***-3'), 26.1 (**C**-4'), 19.8(4) (2-(***C***H_3)(CH_3)), 19.7(9) (2-(CH_3)(***C***H_3)); *m/z* (LCMS, ESI^+) 171 (MH^+); Accurate mass: Found MH^+, 171.1501: $C_9H_{19}N_2O$ requires *M*, 171.1497.

_N_-([Pyrrolidin-2'-yl]methyl)1-methylbenzimidazole-2-carboxamide 193

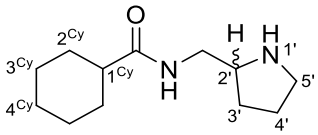


Procedure: 1-Methylbenzimidazole-2-carboxylic acid (35 mg, 0.20 mmol) and 2-(aminomethyl)-1-Boc-pyrrolidine **91** (40 mg, 0.20 mmol) were reacted according to general procedure A.

Following TFA-mediated deprotection and reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (16 mg, 31%) was isolated as an orange oil.

ν_{max} (ATR) 3326 (br), 2954 (w), 2886 (w), 1665 (s), 1536 (s), 1463 (s), 1395 (m), 1332 (m), 1264 (m), 1006 (w) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 8.08 – 8.02 (1H, m, N***H***), 7.77 (1H, dt, *J* = 8.0, 1.0 Hz, 4-***H***), 7.43 (1H, dt, *J* = 8.0, 1.0 Hz, 7-***H***), 7.38 (1H, ddd, *J* = 8.0, 7.0, 1.0 Hz, 6-***H***), 7.33 (1H, ddd, *J* = 8.0, 7.0, 1.0 Hz, 5-***H***), 4.23 (3H, s, NC***H***$_3$), 3.56 (1H, ddd, *J* = 13.5, 6.5, 5.0 Hz, NHC***H***H'), 3.42 – 3.37 (1H, m, 2'-***H***), 3.31 (1H, ddd, *J* = 13.0, 7.5, 5.5 Hz, NHCH***H***'), 3.01 – 2.92 (2H, m, 5'-***H***$_2$), 1.93 (1H, dddd, *J* = 12.5, 9.0, 7.5, 5.5 Hz, 3'-***H***H'), 1.88 – 1.79 (1H, m, 4'-***H***H'), 1.76 – 1.69 (1H, m, 4'-H***H***'), 1.48 (1H, ddt, *J* = 13.0, 9.0, 6.5 Hz, 3'-H***H***'); δ_C (176 MHz, $CDCl_3$) 160.1 (***C***=O), 143.8 (***C***-2), 141.2 (***C***-8), 137.2 (***C***-9), 124.6 (***C***-6), 123.5 (***C***-5), 120.8 (***C***-4), 110.5 (***C***-7), 57.9 (***C***-2'), 46.7 (***C***-5'), 44.1 (NH***C***HH'), 32.2 (N***C***H$_3$), 29.5 (***C***-3'), 25.9 (***C***-4'); *m/z* (LCMS, ESI^+) 259 (MH^+); Accurate mass: Found MH^+, 259.1565: $C_{14}H_{19}N_4O$ requires *M*, 259.1559.

_N_-([Pyrrolidin-2'-yl]methyl)cyclohexanecarboxamide[300] 194

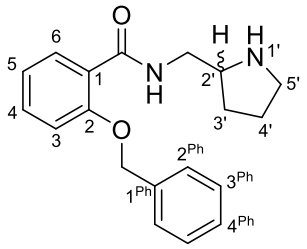


Procedure: Cyclohexanecarboxylic acid (26 mg, 0.20 mmol) and 2-(aminomethyl)-1-Boc-pyrrolidine **91** (40 mg, 0.20 mmol) were reacted according to general procedure A. Following TFA-mediated deprotection and reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the title compound (22 mg, 52%) was isolated as a pale yellow solid.

M.p. 107 – 110 °C; ν_{max} (ATR) 3282 (br), 2927 (s), 2860 (m), 1637 (s), 1542 (s), 1456 (m), 1404 (m), 1256 (w) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 6.12 – 6.03 (1H, m, N***H***), 3.40 – 3.34 (1H, m, NHC***H***H'), 3.24 (1H, dtd, *J* = 8.0, 7.0, 4.5 Hz, 2'-***H***), 3.02 (1H, ddd, *J* = 13.0, 8.0, 5.0 Hz, NHCH***H***'), 2.93 – 2.86 (2H, m, 5'-***H***$_2$), 2.06 (1H, tt, *J* = 12.0, 3.5 Hz, 1^{Cy}-***H***), 1.88 – 1.79 (3H, m, 2^{Cy}-***H***$_2$H'$_2$, 3'-***H***H'),

1.79 – 1.71 (3H, m, 3^{Cy}-***H***$_2$H′$_2$, 4′-***H***H′), 1.71 – 1.62 (2H, m, 4^{Cy}-***H***H′, 4′-H***H***′), 1.41 (2H, tdd, *J* = 12.5, 12.0, 3.0 Hz, 2^{Cy}-H$_2$***H***′$_2$), 1.35 (1H, ddt, *J* = 13.0, 8.5, 7.0 Hz, 3′-H***H***′), 1.29 – 1.15 (3H, m, 3^{Cy}-H$_2$***H***′$_2$, 4^{Cy}-H***H***′); δ_C (176 MHz, $CDCl_3$, mixture of rotamers) 176.4 (***C***=O), 57.9 (***C***-2′), 46.7 (***C***-5′), 45.7 (***C***-1^{Cy}), 43.5 (NH***C***HH′), 29.9(0) (***C***-2^{Cy}), 29.8(5) (***C***-2^{Cy}), 29.2 (***C***-3′), 26.0 (***C***-4′), 25.9(1) (***C***-4^{Cy}), 25.9(0) (***C***-3^{Cy}); *m/z* (LCMS, ESI^+) 211 (MH^+); Accurate mass: Found MH^+, 211.1808: $C_{12}H_{23}N_2O$ requires *M*, 211.1810.

_N_-([Pyrrolidin-2′-yl]methyl)-2-(benzyloxy)benzamide 195

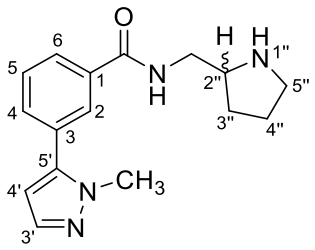


Procedure: 2-(Benzyloxy)benzoic acid (46 mg, 0.20 mmol) and 2-(aminomethyl)-1-Boc-pyrrolidine **91** (40 mg, 0.20 mmol) were reacted according to general procedure A. Following TFA-mediated deprotection and reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (33 mg, 53%) was isolated as an off-white solid.

M.p. 83 – 85 °C; ν_{max} (ATR) 3396 (br), 3058 (w), 2942 (w), 2864 (w), 1643 (s), 1598 (m), 1534 (m), 1482 (m), 1450 (m), 1290 (m), 1230 (m), 1166 (w), 1105 (w), 1006 (w) cm^{-1}; δ_H (600 MHz, $CDCl_3$) 8.21 (1H, dd, *J* = 8.0, 2.0 Hz, 6-***H***), 8.12 – 8.06 (1H, m, N***H***), 7.50 – 7.46 (2H, m, 2^{Ph}-***H***$_2$), 7.45 – 7.35 (4H, m, 4-***H***, 3^{Ph}-***H***$_2$, 4^{Ph}-***H***), 7.09 (1H, td, *J* = 8.0, 1.0 Hz, 5-***H***), 7.05 (1H, dd, *J* = 8.5, 1.0 Hz, 3-***H***), 5.17 (2H, s, OC***H***$_2$), 3.48 (1H, ddd, *J* = 13.0, 6.0, 4.5 Hz, NHC***H***H′), 3.19 (1H, ddd, *J* = 13.0, 7.5, 5.0 Hz, NHCH***H***′), 3.13 (1H, tdd, *J* = 7.5, 7.0, 4.5 Hz, 2′-***H***), 2.81 – 2.72 (2H, m, 5′-***H***$_2$), 1.75 – 1.65 (2H, m, 4′-***H***H′, 3′-***H***H′), 1.64 – 1.56 (1H, m, 4′-H***H***′), 1.30 – 1.22 (1H, m, 3′-H***H***′); δ_C (151 MHz, $CDCl_3$) 165.6 (***C***=O), 157.0 (***C***-2), 135.8 (***C***-1^{Ph}), 132.7 (***C***-4), 132.5 (***C***-6), 129.0 (***C***-3^{Ph}), 128.9 (***C***-4^{Ph}), 128.5 (***C***-2^{Ph}), 122.2 (***C***-1), 121.7 (***C***-5), 112.6 (***C***-3), 71.5 (O***C***H$_2$), 57.8

(***C***-2′), 46.6 (***C***-5′), 44.8 (NH***C***H_2), 29.3 (***C***-3′), 25.8 (***C***-4′); *m/z* (LCMS, ESI^+) 311 (MH^+); Accurate mass: Found MH^+, 311.1757: $C_{19}H_{23}N_2O_2$ requires *M*, 311.1760.

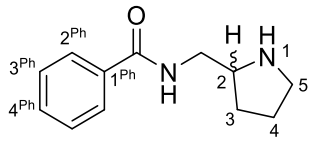
_N_-([Pyrrolidin-2″-yl]methyl)-3-(1′-methylpyrazol-5′-yl)benzamide 196



Procedure: 3-(1′-Methylpyrazol-5′-yl)benzoic acid (40 mg, 0.20 mmol) and 2-(aminomethyl)-1-Boc-pyrrolidine **91** (40 mg, 0.20 mmol) were reacted according to general procedure A. Following TFA-mediated deprotection and reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the *title compound* (34 mg, 60%) was isolated as an orange oil.

ν_{max} (ATR) 3278 (br), 2942 (w), 2876 (w), 1641 (s), 1537 (s), 1476 (w), 1404 (w), 1296 (w), 1188 (w), 1104 (w) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 7.88 (1H, td, *J* = 1.5, 0.5 Hz, 2-***H***), 7.80 (1H, dt, *J* = 7.5, 1.5 Hz, 6-***H***), 7.52 (1H, dt, *J* = 7.5, 1.5 Hz, 4-***H***), 7.52 (1H, d, *J* = 2.0 Hz, 3′-***H***), 7.49 (1H, td, *J* = 7.5, 0.5 Hz, 5-***H***), 7.14 – 7.07 (1H, m, N***H***), 6.34 (1H, d, *J* = 2.0 Hz, 4′-***H***), 3.89 (3H, s, NC***H***$_3$), 3.63 (1H, ddd, *J* = 13.5, 6.0, 4.0 Hz, NHC***H***H′), 3.43 (1H, dddd, *J* = 8.0, 7.5, 7.0, 4.0 Hz, 2″-***H***), 3.23 (1H, ddd, *J* = 13.5, 8.0, 5.0 Hz, NHCH***H***′), 2.96 (1H, ddd, *J* = 11.0, 8.0, 6.0 Hz, 5″-***H***H′), 2.92 (1H, dt, *J* = 11.0, 7.0 Hz, 5″-H***H***′), 1.94 (1H, dddd, *J* = 13.0, 9.0, 7.5, 5.5 Hz, 3″-***H***H′), 1.86 – 1.79 (1H, m, 4″-***H***H′), 1.77 – 1.70 (1H, m, 4″-H***H***′), 1.47 (1H, ddt, *J* = 13.0, 8.5, 7.0 Hz, 3″-H***H***′); δ_C (176 MHz, $CDCl_3$) 167.0 (***C***=O), 142.8 (***C***-5′), 138.8 (***C***-3′), 135.4 (***C***-1), 131.6 (***C***-4), 131.4 (***C***-3), 129.0 (***C***-5), 127.7 (***C***-2), 126.9 (***C***-6), 106.5 (***C***-4′), 57.8 (***C***-2″), 46.6 (***C***-5″), 44.0 (NH***C***HH′), 37.7 (N***C***H_3), 29.3 (***C***-3″), 26.1 (***C***-4″); *m/z* (LCMS, ESI^+) 285 (MH^+); Accurate mass: Found MH^+, 285.1712: $C_{16}H_{21}N_4O$ requires *M*, 285.1715.

***N*-([Pyrrolidin-2-yl]methyl)benzamide[301] 197**



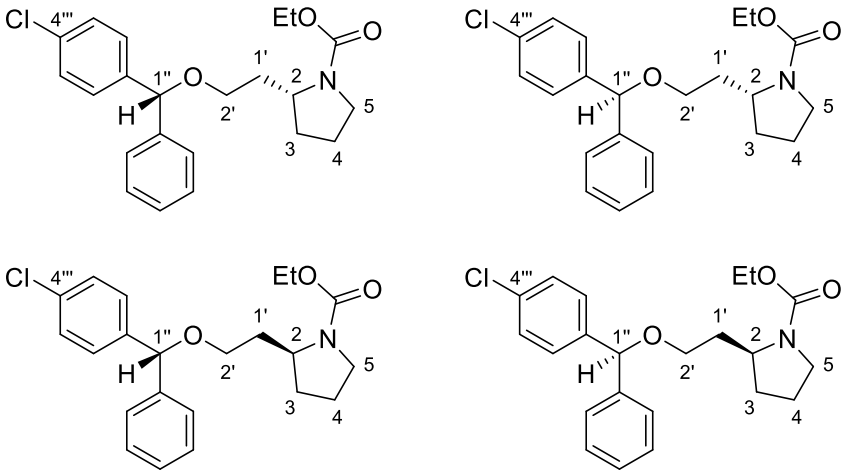
Procedure: Benzoic acid (24 mg, 0.20 mmol) and 2-(aminomethyl)-1-Boc-pyrrolidine **91** (40 mg, 0.20 mmol) were reacted according to general procedure A. Following TFA-mediated deprotection and reversed-phase column chromatography (5% → 95% MeCN in H_2O with 0.5% formic acid), the title compound (7 mg, 17%) was isolated as a yellow oil.

ν_{max} (ATR) 3296 (br), 3054 (w), 2928 (w), 2872 (w), 1636 (s), 1538 (s), 1486 (m), 1404 (m), 1356 (m), 1300 (w) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 7.82 – 7.79 (2H, m, 2^{Ph}-***H***$_2$), 7.50 – 7.45 (1H, m, 4^{Ph}-***H***), 7.44 – 7.39 (2H, m, 3^{Ph}-***H***$_2$), 7.00 – 6.94 (1H, m, N***H***), 3.61 (1H, ddd, *J* = 13.5, 6.0, 4.0 Hz, NHC***H***H'), 3.41 (1H, dddd, *J* = 8.0, 7.5, 7.0, 4.0 Hz, 2-***H***), 3.23 (1H, ddd, *J* = 13.5, 8.0, 5.0 Hz, NHCH***H***'), 2.96 – 2.89 (2H, m, 5-***H***$_2$), 1.91 (1H, dddd, *J* = 13.0, 9.0, 7.5, 5.5 Hz, 3-***H***H'), 1.84 – 1.78 (1H, m, 4-***H***H'), 1.75 – 1.68 (1H, m, 4-H***H***'), 1.46 (1H, ddt, *J* = 13.0, 8.5, 7.0 Hz, 3-H***H***'); δ_C (176 MHz, $CDCl_3$) 167.7 (***C***=O), 134.8 (***C***-1^{Ph}), 131.5 (***C***-4^{Ph}), 128.6 (***C***-3^{Ph}), 127.1 (***C***-2^{Ph}), 57.9 (***C***-2), 46.6 (***C***-5), 44.0 (NH***C***HH'), 29.3 (***C***-3), 26.1 (***C***-4); *m/z* (LCMS, ESI^+) 205 (MH^+); Accurate mass: Found MH^+, 205.1347: $C_{12}H_{17}N_2O$ requires *M*, 205.1341.

8.4 Additional compounds

2-(2′-[{4‴-Chlorophenyl}{phenyl}methoxy]ethyl)-1-(ethoxycarbonyl)pyrrolidine 198

A mixture of (2*R*,1″*R*), (2*R*,1″*S*), (2*S*,1″*R*) and (2*S*,1″*S*) diastereomers

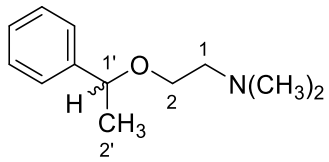


Procedure:[302] Ethyl chloroformate (15 µL, 0.15 mmol) was added dropwise to an ice cold solution of amine **77** (43 mg, 0.14 mmol), NEt_3 (21 µL, 0.15 mmol) and DCM (0.4 mL). After allowing the reaction mixture to warm slowly to RT over 2.5 h, TLC analysis showed consumption of amine **77**. Ethyl acetate (5 mL) was then added and the solids removed by filtration through celite®, followed by washing the filter cake with ethyl acetate (3 × 5 mL). The combined organic filtrates were then concentrated *in vacuo* prior to purification by chromatography on SiO_2 (15% → 30% EtOAc in hexanes) to afford the *title compound* (25 mg, 46%) as a clear colourless oil.

R_f 0.70 (50% EtOAc in hexanes); ν_{max} (ATR) 2960 (w), 2870 (w), 1689 (s), 1488(w), 1414 (m), 1379 (w), 1333 (w), 1184 (w), 1088 (s) cm^{-1}; δ_H (700 MHz, $CDCl_3$,mixture of diastereomers) 7.33 – 7.20 (9H, m, Ar***H***), 5.33 – 5.28 (1H, m, 1′′-***H***), 4.15 – 4.05 (2H, m, OC***H***$_2$$CH_3$), 3.99 – 3.93 (1H, m, 2-***H***), 3.52 – 3.27 (4H, m, 2′-***H***$_2$, 5-***H***$_2$), 2.21 – 2.04 (1H, m, 1′-***H***H′), 1.95 – 1.87 (1H, m, 3-***H***H′), 1.87 – 1.77 (2H, m, 4-***H***$_2$), 1.76 – 1.71 (1H, m, 3-H***H***′), 1.66 – 1.58 (1H, m, 1′-H***H***′), 1.26 – 1.18 (3H, m, OCH_2C***H***$_3$); δ_C (176 MHz, $CDCl_3$, mixture of diastereomers) 155.2 (***C***=O), 142.1 (Ar***C***), 141.3 (Ar***C***), 133.2 (Ar***C***), 128.5(8) (Ar***C***), 128.5(6) (Ar***C***), 128.4 (Ar***C***), 127.7 (Ar***C***), 127.0 (Ar***C***), 83.0 (***C***-1′′), 66.9 (***C***-2′), 66.8 (***C***-2′), 60.9 (O***C***H_2CH_3), 60.8 (O***C***H_2CH_3), 56.0 (***C***-2), 55.2 (***C***-2), 46.5 (***C***-5), 46.3 (***C***-5), 34.9 (***C***-1′), 34.2 (***C***-1′), 31.2 (***C***-3), 30.7 (***C***-3), 24.0 (***C***-4), 23.2 (***C***-4), 14.9

($OCH_2\boldsymbol{C}H_3$); *m/z* (LCMS, ESI^+) 388 ($M(^{35}Cl)H^+$), 390 ($M(^{37}Cl)H^+$); Accurate mass: Found MH^+, 388.1681: $C_{22}H_{27}NO_3{}^{35}Cl$ requires *M*, 388.1679.

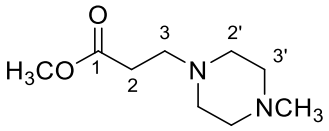
2-(1'-Phenylethoxy)-ethyldimethylamine 199



Procedure: To a solution of 2-dimethylaminoethanol **81** (0.50 mL, 5 mmol) in DCM (10 mL) was added methanesulfonyl chloride (0.41 mL, 5.25 mmol) and the mixture stirred at RT until TLC analysis showed consumption of starting material. The reaction was then diluted with DCM (5 mL) before the addition of 1-phenylethanol **79** (0.62 mL, 5 mmol) and KO^tBu (1.17g, 10.25 mmol). After stirring for 4 h the reaction was quenched with the addition of H_2O (15 mL) and the biphasic mixture was extracted with DCM (3 × 10 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated *in vacuo* before purification by SiO_2 column (0% → 10% MeOH in $CHCl_3$ with 1% NEt_3) furnished the *title compound* (628 mg, 65%) as a clear colourless oil.

R_f 0.23 (10% MeOH and 1% NEt_3 in $CHCl_3$); ν_{max} (ATR) 2973 (w), 2929 (w), 2853 (w), 2813 (w), 2764 (w), 1446 (m), 1276 (w), 1104 (s), 1032 (m) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 7.33 – 7.29 (4H, m, Ar***H***), 7.26 – 7.23 (1H, m, Ar***H***), 4.39 (1H, q, *J* = 6.5 Hz, 1'-***H***), 3.39 (2H, t, *J* = 6.0 Hz, 2-$\boldsymbol{H}_2$), 2.54 – 2.47 (2H, m, 1-$\boldsymbol{H}_2$), 2.24 (6H, s, $N(C\boldsymbol{H}_3)_2$), 1.43 (3H, d, *J* = 6.5 Hz, 2'-$\boldsymbol{H}_3$); δ_C (176 MHz, $CDCl_3$) 143.9 (***C***-5), 128.5 (Ar***C***), 127.5 (Ar***C***), 126.3 (Ar***C***), 78.6 (***C***-1'), 66.6 (***C***-2), 59.1 (***C***-1), 45.8 ($N(\boldsymbol{C}H_3)_2$), 24.2 (***C***-2'); *m/z* (LCMS, ESI^+) 194 (MH^+); Accurate mass: Found MH^+, 194.1524: $C_{12}H_{20}NO$ requires *M*, 194.1545.

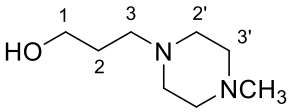
Methyl 3-(4′-methylpiperazin-1′-yl)propanoate[303] 200



Procedure:[304] A solution of 1-methylpiperazine (1.12 mL, 10.00 mmol), methyl acrylate (1.00 mL, 11.00 mmol) and I_2 (838 mg, 3.30 mmol) in DCM (10 mL) was stirred at RT for 18 h. The reaction mixture was then washed with saturated aq. sodium thiosulfate (2 × 5 mL), dried over $MgSO_4$ and the volatiles removed under reduced pressure. Purification by column chromatography (0% → 10% MeOH in $CHCl_3$ with 1% NEt_3) gave the title compound (1.20 g, 64%) as a yellow oil.

R_f 0.53 (10% MeOH in $CHCl_3$ with 1% NEt_3); ν_{max} (ATR) 3360 (br), 2940 (m), 2795 (m), 1733 (s), 1438 (m), 1282 (m), 1161 (s), 1085 (w), 1010 (s) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 3.65 (3H, s, OC***H***$_3$), 2.68 (2H, t, *J* = 7.5 Hz, 2-***H***$_2$), 2.66 – 2.31 (8H, m, 2′-(***H***$_2$)$_2$, 3′-(***H***$_2$)$_2$), 2.48 (2H, t, *J* = 7.5 Hz, 3-***H***$_2$), 2.26 (3H, s, NC***H***$_3$); δ_C (176 MHz, $CDCl_3$) 173.0 (***C***=O), 55.1 (***C***-3′), 53.6 (***C***-2), 52.9 (***C***-2′), 51.7 (O***C***H_3), 46.0 (N***C***H_3), 32.2 (***C***-3); m/*z* (LCMS, ESI^+) 187 (MH^+).

3-(4′-Methylpiperazin-1′-yl)propan-1-ol[305] 201

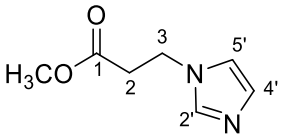


Procedure:[222] To a 0 °C solution of methyl ester **200** (1.00 g, 5.40 mmol) in THF (27 mL) was slowly added $LiAlH_4$ (2.4 M solution in THF, 3.40 mL, 8.10 mmol), before being allowed to warm to RT. After 3 h the mixture was cooled once more in an ice bath and quenched according to Fieser's method (general procedure D).[273] The combined organic filtrates were concentrated *in vacuo* to afford the crude product (715 mg, 84%) as a clear colourless oil which was used without any further purification.

R_f 0.29 (10% MeOH in $CHCl_3$ with 1% $NH_4OH_{(aq.)}$); ν_{max} (ATR) 3324 (br), 2940 (m), 2809 (m), 1649 (w), 1459 (m), 1283 (m), 1147 (m), 1009 (m) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 3.75 – 3.73 (2H, m, 1-***H***$_2$), 2.84 – 2.12 (8H, m, 2′-(***H***$_2$)$_2$, 3′-(***H***$_2$)$_2$), 2.58 – 2.56 (2H, m, 3-***H***$_2$), 2.23 (3H, s, NC***H***$_3$), 1.69 –

1.65 (2H, m, 2-***H***$_2$); δ_C (176 MHz, $CDCl_3$) 64.5 (***C***-1), 58.7 (***C***-3), 55.2 (***C***-3′), 53.3 (***C***-2′), 46.0 (N***C***H$_3$), 27.2 (***C***-2); m/*z* (LCMS, ESI$^+$) 159 (MH^+).

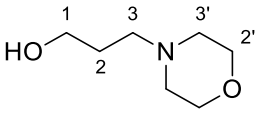
Methyl 3-(imidazol-1′-yl)propanoate[306] 202



Procedure:[304] Imidazole (681 mg, 10.00 mmol) was reacted with methyl acrylate (1.00 mL, 11.00 mmol) according to the conditions for the synthesis of ester **200**, providing the title compound (161 mg, 10%) as a pale yellow oil.

R_f 0.50 (10% MeOH in $CHCl_3$ with 1% NEt_3); ν_{max} (ATR) 3306 (br), 3110 (w), 2954 (w), 1728 (s), 1508 (m), 1438 (m), 1208 (s), 1168 (s), 1080 (m) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 7.52 (1H, s, 2′-***H***), 7.02 (1H, s, 4′-***H***), 6.92 (1H, s, 5′-***H***), 4.26 (2H, t, *J* = 6.5 Hz, 3-***H***$_2$), 3.67 (3H, s, OC***H***$_3$), 2.77 (2H, t, *J* = 6.5 Hz, 2-***H***$_2$); δ_C (176 MHz, $CDCl_3$) 171.0 (***C***=O), 137.3 (***C***-2′), 129.5 (***C***-4′), 119.0 (***C***-5′), 52.2 (O***C***H$_3$), 42.4 (***C***-3), 35.9 (***C***-2); m/*z* (LCMS, ESI$^+$) 155 (MH^+).

3-(Morpholin-4′-yl)propan-1-ol[307] 203

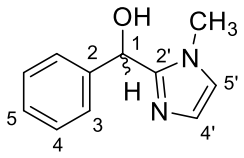


Procedure: A mixture of 3-bromo-1-propanol (1.40 mL, 15.00 mmol), morpholine (3.28 mL, 37.50 mmol) and potassium iodide (30 mg, 0.18 mmol) in DCM (23 mL) was stirred at RT for 21 h. 1 M $NaOH_{(aq.)}$ (16 mL) was then added and the biphasic mixture extracted with DCM (3 × 10 mL), after which the combined organic extracts were dried over $MgSO_4$ and concentrated *in vacuo*. Purification by SiO_2 column (0% → 10% MeOH in $CHCl_3$ with 1% NEt_3) afforded the title compound (928 mg, 43%) as a pale yellow oil.

R_f 0.63 (10% MeOH in $CHCl_3$ with 1% NEt_3); ν_{max} (ATR) 3356 (br), 2942 (m), 2853 (m), 2812 (m), 1447 (w), 1303 (w), 1269 (w), 1113 (s), 1065 (m) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 3.78 (2H, t,

J = 5.5 Hz, 1-***H***$_2$), 3.72 – 3.66 (4H, m, 2′-(***H***$_2$)$_2$), 2.61 (2H, t, *J* = 6.0 Hz, 3-***H***$_2$), 2.56 – 2.40 (4H, m, 3′-(***H***$_2$)$_2$), 1.74 – 1.70 (2H, m, 2-***H***$_2$); δ_C (176 MHz, $CDCl_3$) 67.0 (***C***-2′), 64.5 (***C***-1), 59.2 (***C***-3), 53.9 (***C***-3′), 26.9 (***C***-2); *m/z* (LCMS, ESI^+) 146 (MH^+).

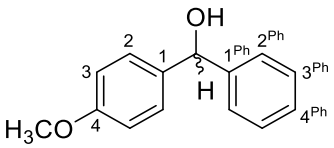
Phenyl(1′-methylimidazol-2′-yl)methanol[308] 204



Procedure:[309] A solution of *N*-methyl imidazole (0.56 mL, 7.00 mmol), in THF (7 mL) was cooled to −78 °C and *n*-BuLi (1.6 M in hexanes, 4.33 mL, 7.00 mmol) was added dropwise. The mixture was stirred for 15 min before being warmed to −20 °C for 1 h. After being cooled once more to −78 °C, a solution of benzaldehyde (0.71 mL, 7.00 mmol) in THF (4 mL) was added *via* cannula and the reaction mixture allowed to warm slowly to RT. After TLC analysis showed consumption of benzaldehyde, the reaction was quenched by the addition of 3 M $HCl_{(aq.)}$ (15 mL) and THF removed under reduced pressure. Ether (20 mL) was added, the mixture was separated and the organic phase washed with 3 M $HCl_{(aq.)}$ (3 × 15 mL). The collected aqueous washings were then adjusted to pH ≈ 9 and extracted with DCM (3 × 30 mL). The combined organic fractions were dried over $MgSO_4$ and concentrated *in vacuo* to afford the title compound (928 mg, 70%) as an off-white solid that was used without further purification.

R_f 0.57 (10% MeOH in $CHCl_3$ with 1% $NH_4OH_{(aq.)}$); ν_{max} (ATR) 3076 (br), 2822 (m), 2680 (m), 1492 (m), 1446 (m), 1406 (w), 1272 (m), 1174 (w), 1134 (w), 1049 (m), 1020 (m) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 7.34 – 7.30 (4H, m, Ar***H***), 7.27 – 7.24 (1H, m, Ar***H***), 6.87 – 6.86 (1H, m, 4′-***H***), 6.74 – 6.73 (1H, m, 5′-***H***), 5.92 (1H, s, 1-***H***), 3.38 (3H, s, NC***H***$_3$); δ_C (151 MHz, $CDCl_3$) 149.3 (***C***-2′), 141.0 (***C***-2), 128.5 (Ar***C***), 127.7 (Ar***C***), 126.4(1) (***C***-4′), 126.3(8) (***C***-3), 122.3 (***C***-5′), 69.2 (***C***-1), 33.3 (N***C***H$_3$); *m/z* (LCMS, ESI^+) 189 (MH^+).

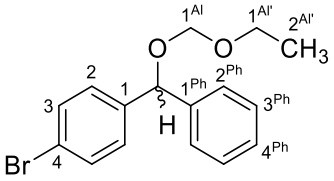
4-Methoxyphenyl(phenyl)methanol[310] 205



Procedure: 4-Methoxybenzaldehyde (99%, 2.13 mL, 20.00 mmol) was reacted according to general procedure C, furnishing the title compound (2.57 g, quantitative) as an off-white solid after chromatography on silica (0% → 60% ether in hexanes).

R_f 0.31 (40% ether in hexanes); M.p. 63 – 65 °C (lit.:[311] 62 – 65 °C); ν_{max} (ATR) 3552 (br), 3056 (w), 3029 (w), 3001 (w), 2956 (w), 1511 (s), 1455 (w), 1366 (s), 1230 (s), 1032 (m) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 7.39 – 7.27 (7H, m, Ar***H***), 6.89 – 6.85 (2H, m, 3-***H***$_2$), 5.82 (1H, s, C***H***OH), 3.79 (3H, s, OC***H***$_3$); δ_C (176 MHz, $CDCl_3$) 159.2 (***C***-4), 144.2 (***C***-1^{Ph}), 136.3 (***C***-3), 128.6 (Ar***C***), 128.1 (Ar***C***), 127.6 (Ar***C***), 126.5 (Ar***C***), 114.0 (***C***-3), 76.0 (***C***HOH), 55.4 (O***C***H_3); *m/z* (LCMS, ESI^+) 197 ($M - OH^-$).

4-Bromophenyl(phenyl)(ethoxymethoxy)methane 206

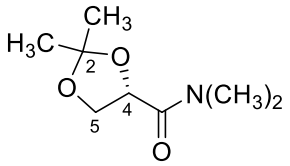


Procedure: To a 0 °C solution of alcohol **164** (1.01 g, 3.85 mmol) in DCM (10 mL) was added DIPEA (99%, 1.35 mL, 7.69 mmol) then chloromethylethyl ether (95%, 0.64 mL, 6.55 mmol). The mixture was reacted at 40 °C overnight, after which full conversion of starting material was observed by TLC analysis. Saturated $NH_4Cl_{(aq.)}$ (10 mL) was added and the aqueous layer removed and washed with DCM (3 × 10 mL). The combined organic extracts were dried over $MgSO_4$, concentrated *in vacuo*, and the residue purified *via* silica flash column chromatography (0% → 40% ether in hexanes) to provide the *title compound* (996 mg, 80%) as a clear yellow oil.

R_f 0.63 (40% ether in hexanes); ν_{max} (ATR) 3017 (w), 2943 (w), 2882 (w), 1486 (m), 1455 (m), 1366 (s), 1218 (s), 1097 (m), 1008 (s) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 7.46 – 7.44 (2H, m, 3-***H***$_2$), 7.33 (4H, apparent d, *J* = 4.5 Hz, 2^{Ph}-***H***$_2$, 3^{Ph}-***H***$_2$), 7.29 – 7.26 (1H, m, 4^{Ph}-***H***), 7.26 – 7.23 (2H, m, 2-***H***$_2$),

5.71 (1H, s, $Ar_2C\boldsymbol{H}$), 4.73 (2H, apparent d, *J* = 1.5 Hz, 1^{Al}-$\boldsymbol{H}_2$), 3.64 (2H, apparent dq, *J* = 7.0, 1.0 Hz, $1^{Al'}$-$\boldsymbol{H}_2$), 1.18 (3H, t, *J* = 7.0 Hz, $2^{Al'}$-$\boldsymbol{H}_3$); δ_C (176 MHz, $CDCl_3$) 141.5 (***C***-1^{Ph}), 141.3 (***C***-1), 131.6 (***C***-3), 129.0 (***C***-2), 128.6 (***C***-3^{Ph}), 127.8 (***C***-4^{Ph}), 127.3 (***C***-2^{Ph}), 121.5 (***C***-4), 92.9 (***C***-1^{Al}), 78.4 ($Al_2\boldsymbol{C}H$), 63.8 (***C***-$1^{Al'}$), 15.2 (***C***-$2^{Al'}$); *m/z* (LCMS, ESI^+) 245 ($M(^{79}Br) - C_3H_7O_2^-$), 247 ($M(^{81}Br) - C_3H_7O_2^-$).

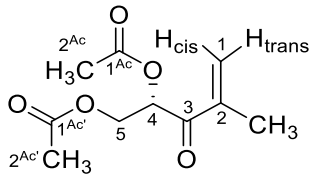
(4*S*)-*N*,*N*,2,2-Tetramethyl-1,3-dioxolane-4-carboxamide[312] 207



Procedure:[312] (−)-Methyl (4*S*)-2,2-dimethyl-1,3-dioxolane-4-carboxylate (96%, 2.65 g, 15.90 mmol) was added to a 0 °C solution of dimethylamine (2 M in MeOH, 80 mL). The mixture was stirred at RT for 24 h, before the removal of volatiles *in vacuo* provided a residue which was purified on SiO_2 column (0% → 50% EtOAc in hexanes). The title compound (2.53 g, 92%) was isolated as a clear colourless oil.

No optical rotation measurement was obtained. R_f 0.18 (40% EtOAc in hexanes); ν_{max} (ATR) 2980 (w), 2934 (w), 2870 (w), 1647 (s), 1498 (w), 1372 (m), 1256 (m), 1213 (m), 1151 (m), 1064 (s) cm^{-1}; δ_H (600 MHz, $CDCl_3$) 4.68 (1H, t, *J* = 6.5 Hz, 4-***H***), 4.37 (1H, dd, *J* = 8.5, 6.5 Hz, 5-***H***H'), 4.13 (1H, dd, *J* = 8.5, 6.5 Hz, 5-H***H***'), 3.11 (3H, s, $N(C\boldsymbol{H}_3)CH_3$), 2.96 (3H, s, $N(CH_3)C\boldsymbol{H}_3$), 1.42 – 1.39 (6H, m, 2-$(C\boldsymbol{H}_3)_2$); δ_C (151 MHz, $CDCl_3$) 169.1 (***C***=O), 110.6 (***C***-2), 73.6 (***C***-4), 66.6 (***C***-5), 37.0 ($N(\boldsymbol{C}H_3)CH_3$), 35.9 ($N(CH_3)\boldsymbol{C}H_3$), 26.1 (2-($\boldsymbol{C}H_3$)(CH_3)), 25.9 (2-(CH_3)($\boldsymbol{C}H_3$)); *m/z* (LCMS, ESI^+) 174 (MH^+), 196 (MNa^+).

(4*S*)-2-methyl-3-oxopent-1-ene-4,5-diyl diacetate[313] 208

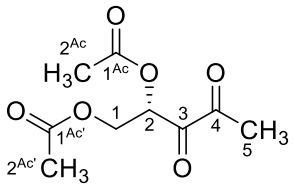


Procedure:[312,313] Isopropenylmagnesium bromide (0.5 M in THF, 30.30 mL, 15.20 mmol) was added to a stirred 0 °C solution of amide **207** (2.50 g, 14.50 mmol) in ether (60 mL). After 20 min, when TLC analysis revealed consumption of starting material, the solution was cooled in an ice bath and 1 M HCl (40 mL) was added. The biphasic mixture was separated and the aqueous layer washed with ether (2 × 20 mL) and brine (40 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated *in vacuo*, before purification by SiO_2 column (0% → 20% ether in hexanes) provided 1-((4′*S*)-2′,2′-dimethyl-1′,3′-dioxolan-4′-yl)-2-methylprop-2-en-1-one (789 mg, 32%) as a clear colourless oil.

The prepared dioxolane proved to be unstable, so was carried forward immediately to the next step in the sequence. 1-((4′*S*)-2′,2′-dimethyl-1′,3′-dioxolan-4′-yl)-2-methylprop-2-en-1-one (105 mg, 0.62 mmol) was dissolved in 90% $MeOH_{(aq.)}$ (2 mL) and stirred at reflux with DOWEX ion exchange resin (50WX2 100-200 mesh, 105 mg) for 1.5 h. The mixture was then filtered and concentrated under reduced pressure before being dissolved in DCM (2.5 mL) and cooled to 0 °C. Pyridine (0.11 mL, 1.36 mmol), NEt_3 (99%, 8 µL, 0.06 mmol) then acetic anhydride (0.29 mL, 3.1 mmol) were added and the reaction stirred at RT. After 5 h the volatiles were removed *in vacuo* and the residue separated on silica column (0% → 50% EtOAc in hexanes) to furnish the title compound (101 mg, 81%) as a clear colourless oil.

No optical rotation measurement was obtained. R_f 0.52 (50% EtOAc in hexanes); ν_{max} (ATR) 2976 (w), 2934 (w), 1737 (s), 1693 (m), 1440 (w), 1371 (m), 1216 (s), 1044 (m) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 6.12 (1H, q, *J* = 1.0 Hz, 1-***H***$_{trans}$), 5.95 (1H, q, *J* = 1.5 Hz, 1-***H***$_{cis}$), 5.90 (1H, dd, *J* = 7.0, 3.0 Hz, 4-***H***), 4.51 (1H, dd, *J* = 12.0, 3.0 Hz, 5-***H***H′), 4.26 (1H, dd, *J* = 12.0, 7.0 Hz, 5-H***H***′), 2.16 (3H, s, 2^{Ac}-***H***$_3$), 2.08 (3H, s, $2^{Ac'}$-***H***$_3$), 1.91 (3H, dd, *J* = 1.5, 1.0 Hz, 2-C***H***$_3$); δ_C (176 MHz, $CDCl_3$) 194.8 (***C***-3), 170.8 (***C***-$1^{Ac'}$), 170.3 (***C***-1^{Ac}), 142.5 (***C***-2), 126.6 (***C***-1), 72.8 (***C***-4), 63.2 (***C***-5), 20.9 (***C***-$2^{Ac'}$), 20.8 (***C***-2^{Ac}), 17.9 (4-***C***H_3); *m/z* (GCMS, EI) 43 ($CH_3CO^{\bullet}$), 171 (M − $CH_3CO^{\bullet}$).

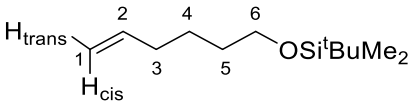
(2*S*)-3,4-dioxopentane-1,2-diyl diacetate[313] 209



Procedure:[313] A solution of olefin **208** (155 mg, 0.77 mmol) in MeOH (25 mL) was cooled to −78 °C and a mixture of O_3 in O_2 was bubbled through the stirred solution until a persistent blue colour was achieved. The solution was then purged with O_2 and dimethylsulfide (0.80 mL, 10.84 mmol) was added dropwise. The quenched mixture was warmed to RT overnight, after which the solution displayed minimal peroxide content. After concentration under reduced pressure, the residue was purified by SiO_2 flash chromatography (0% → 40% ether in hexanes) to furnish the title compound (83 mg, 50%) as a yellow oil.

No optical rotation measurement was obtained. R_f 0.18 (20% EtOAc in hexanes); ν_{max} (ATR) 2994 (w), 2924 (w), 1732 (s), 1438 (w), 1373 (m), 1218 (s), 1038 (m) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 5.84 (1H, t, *J* = 4.0 Hz, 2-***H***), 4.67 (1H, dd, *J* = 12.5, 4.0 Hz, 1-***H***H′), 4.34 (1H, dd, *J* = 12.5, 4.0 Hz, 1-H***H***′), 2.38 (3H, s, 5-$\boldsymbol{H}_3$), 2.16 (3H, s, 2^{Ac}-$\boldsymbol{H}_3$), 2.04 (3H, s, $2^{Ac'}$-$\boldsymbol{H}_3$); δ_C (176 MHz, $CDCl_3$) 196.0 (***C***-4), 190.2 (***C***-3), 171.1 (***C***-$1^{Ac'}$), 170.2 (***C***-1^{Ac}), 72.8 (***C***-2), 62.5 (***C***-1), 23.8 (***C***-5), 20.7 (***C***-$2^{Ac'}$), 20.5 (***C***-2^{Ac}); *m/z* (GCMS, EI) 43 ($CH_3CO^{\bullet}$), 173 (M − $CH_3CO^{\bullet}$).

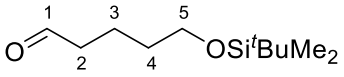
6-(t*ert*-Butyldimethylsilyloxy)hex-1-ene[314] 210



Procedure: To a mixture of 5-hexen-1-ol (98%, 304 mg, 2.97 mmol), NEt_3 (99%, 0.50 mL, 3.56 mmol) and 4-dimethylaminopyridine (99%, 74 mg, 0.60 mmol) in DCM (3 mL) was added a solution of *tert*-butyldimethylsilyl chloride (97%, 485 mg, 3.12 mmol) in DCM (2 mL). The mixture was stirred for 4 h, after which TLC analysis revealed consumption of the reagent alcohol. The reaction was quenched by the addition of H_2O (8 mL) and the mixture passed through a phase separator. After removal of volatiles, the organic residue was purified *via* silica column chromatography (0% → 20% ether in hexanes) to afford the title compound (490 mg, 77%) as a clear colourless oil.

R_f 0.79 (20% EtOAc in hexanes); ν_{max} (ATR) 2954 (m), 2928 (m), 2861 (m), 1258 (s), 1032 (br, s) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 5.81 (1H, ddt, *J* = 17.0, 10.0, 6.5 Hz, 2-***H***), 5.00 (1H, ddt, *J* = 17.0, 2.0, 1.5 Hz, 1-$\boldsymbol{H}_{cis}$), 4.94 (1H, ddt, *J* = 10.0, 2.0, 1.0 Hz, 1-$\boldsymbol{H}_{trans}$), 3.61 (2H, t, *J* = 6.5 Hz, 6-$\boldsymbol{H}_2$), 2.10 – 2.03 (2H, m, 3-$\boldsymbol{H}_2$), 1.57 – 1.50 (2H, m, 5-$\boldsymbol{H}_2$), 1.47 – 1.39 (2H, m, 4-$\boldsymbol{H}_2$), 0.89 (9H, s, OSi(C(C$\boldsymbol{H}_3$)$_3$)(CH$_3$)$_2$), 0.05 (6H, s, OSi(C(CH$_3$)$_3$)(C$\boldsymbol{H}_3$)$_2$); δ_C (176 MHz, $CDCl_3$) 139.1 (***C***-2), 114.5 (***C***-1), 63.2 (***C***-6), 33.7 (***C***-3), 32.5 (***C***-5), 26.1 (OSi(C(***C***H$_3$)$_3$)(CH$_3$)$_2$), 25.3 (***C***-4), 18.5 (OSi(***C***(CH$_3$)$_3$)(CH$_3$)$_2$), −5.1 (OSi(C(CH$_3$)$_3$)(***C***H$_3$)$_2$); *m/z* (GCMS, EI) 157 (M − C(CH$_3$)$_3^{\bullet}$), 199 (M − CH$_3^{\bullet}$).

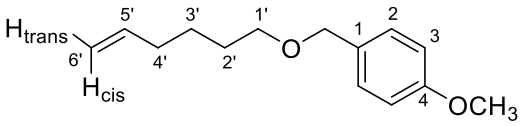
5-(*tert*-Butyldimethylsilyloxy)pentanal[315] 211



Procedure:[313] A solution of olefin **210** (429 mg, 2.00 mmol) in MeOH (20 mL) was cooled to −78 °C and a mixture of O_3 in O_2 was bubbled through the stirred solution until a persistent blue colour was achieved. The solution was then purged with O_2 and dimethylsulfide (2.06 mL, 28.00 mmol) was added dropwise. The quenched mixture was warmed to RT overnight, after which the solution displayed minimal peroxide content. After concentration under reduced pressure, the residue was purified by SiO_2 flash chromatography (0% → 40% EtOAc in hexanes) to furnish the title compound (262 mg, 61%) as a clear colourless oil.

R_f 0.22 (20% ether in hexanes); ν_{max} (ATR) 2954 (w), 2929 (w), 2858 (w), 1729 (s), 1468 (w), 1410 (w), 1253 (m), 1095 (s) cm^{-1}; δ_H (600 MHz, $CDCl_3$) 9.78 – 9.76 (1H, m, 1-***H***), 3.63 (2H, t, *J* = 6.0 Hz, 5-$\boldsymbol{H}_2$), 2.48 – 2.44 (2H, m, 2-$\boldsymbol{H}_2$), 1.74 – 1.67 (2H, m, 3-$\boldsymbol{H}_2$), 1.60 – 1.52 (2H, m, 4-$\boldsymbol{H}_2$), 0.89 (9H, s, OSi(C(C$\boldsymbol{H}_3$)$_3$)(CH$_3$)$_2$), 0.04 (6H, s, OSi(C(CH$_3$)$_3$)(C$\boldsymbol{H}_3$)$_2$); δ_C (151 MHz, $CDCl_3$) 202.9 (***C***-1), 62.8 (***C***-5), 43.8 (***C***-2), 32.3 (***C***-4), 26.1 (OSi(C(***C***H$_3$)$_3$)(CH$_3$)$_2$), 18.8 (***C***-3), 18.5 (OSi(***C***(CH$_3$)$_3$)(CH$_3$)$_2$), −5.2 (OSi(C(CH$_3$)$_3$)(***C***H$_3$)$_2$); *m/z* (LCMS, ESI$^+$) 217 (MH$^+$); Accurate mass: Found MH$^+$, 217.1635: $C_{11}H_{25}O_2Si$ requires *M*, 217.1624.

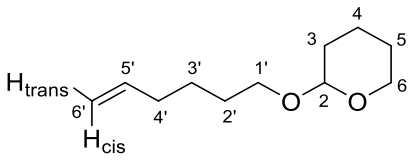
1-([{Hex-5′-en-1′-yl}oxy]methyl)-4-methoxybenzene[316] 212



Procedure:[316] To a 0 °C solution of NaH (60%, 424 mg, 10.61 mmol) in DMF (8 mL) was added 5-hexen-1-ol (98%, 1.00 mL, 8.16 mmol) in DMF (20 mL). After stirring in an ice bath for 1 h, 4-methoxybenzyl chloride (98%, 1.35 mL, 9.79 mmol) was added dropwise and the reaction mixture warmed to RT. After 5 h, the mixture was re-cooled to 0 °C and quenched by the addition of ice cold H_2O (200 mL). The biphasic mixture was extracted with hexanes (6 × 20 mL) and the collected organic washings concentrated *in vacuo* then purified on SiO_2 column (0% → 20% ether in hexanes) to furnish the title compound (1.47 g, 82%) as a clear colourless oil.

R_f 0.28 (5% ether in hexanes); ν_{max} (ATR) 3000 (w), 2936 (w), 2857 (w), 1613 (w), 1512 (s), 1455 (w), 1366 (m), 1302 (w), 1245 (s), 1097 (s), 1036 (s) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 7.29 – 7.24 (2H, m, 2-***H***$_2$), 6.90 – 6.85 (2H, m, 3-***H***$_2$), 5.80 (1H, ddt, *J* = 17.0, 10.0, 6.5 Hz, 5′-***H***), 5.00 (1H, dq, *J* = 17.0, 2.0, 1.5 Hz, 6′-***H***$_{cis}$), 4.94 (1H, ddt, *J* = 10.0, 2.0, 1.0 Hz, 6′-***H***$_{trans}$), 4.43 (2H, s, OC***H***$_2$), 3.80 (3H, s, OC***H***$_3$), 3.45 (2H, t, *J* = 6.5 Hz, 1′-***H***$_2$), 2.06 (2H, tddd, *J* = 8.0, 6.5, 1.5, 1.0 Hz, 4′-***H***$_2$), 1.65 – 1.58 (2H, m, 2′-***H***$_2$), 1.50 – 1.43 (2H, m, 3′-***H***$_2$); δ_C (176 MHz, $CDCl_3$) 159.3 (***C***-4), 138.9 (***C***-5′), 130.9 (***C***-1), 129.4 (***C***-2), 114.6 (***C***-6′), 113.9 (***C***-3), 72.7 (O***C***H_2), 70.1 (***C***-1′), 55.4 (O***C***H_3), 33.7 (***C***-4′), 29.4 (***C***-2′), 25.7 (***C***-3′); *m/z* (GCMS, EI) 121 (CH_2([*p*-OCH_3]C_6H_4)$^•$), 220 ($M^•$).

2-([Hex-5′-en-1′-yl]oxy)tetrahydropyran[317] 213

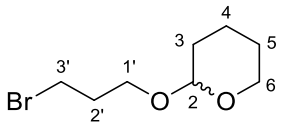


Procedure: A mixture of 5-hexen-1-ol (98%, 1.00 mL, 8.16 mmol), 3,4-dihydro-2*H*-pyran (97%, 1.14 mL, 12.24 mmol) and pyridinium *p*-toluenesulfonate (98%, 210 mg, 0.82 mmol) was stirred in DCM (30 mL) for 5 h. H_2O (20 mL) was added to quench the reaction before the mixture was passed through a phase separator. Subsequent removal of volatiles and

purification of the organic residue by SiO_2 column chromatography (0% → 20% ether in hexanes) provided the title compound (1.38 g, 92%) as a colourless clear oil.

R_f 0.50 (20% ether in hexanes); ν_{max} (ATR) 2939 (m), 2869 (m), 1441 (w), 1353 (w), 1201 (m), 1119 (m), 1078 (m), 1033 (s) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 5.81 (1H, ddt, *J* = 17.0, 10.0, 6.5 Hz, 5'-***H***), 5.00 (1H, ddt, *J* = 17.0, 2.0, 1.5 Hz, 6'-$\boldsymbol{H}_{cis}$), 4.94 (1H, ddt, *J* = 10.0, 2.0, 1.0 Hz, 6'-$\boldsymbol{H}_{trans}$), 4.57 (1H, dd, *J* = 4.5, 3.0 Hz, 2-***H***), 3.86 (1H, ddd, *J* = 11.0, 8.0, 3.0 Hz, 6-***H***H'), 3.73 (1H, dt, *J* = 9.5, 7.0 Hz, 1'-***H***H'), 3.52 – 3.46 (1H, m, 6-H***H***'), 3.38 (1H, dt, *J* = 9.5, 6.5 Hz, 1'-H***H***'), 2.08 (2H, tddd, *J* = 7.5, 6.5, 1.5, 1.0 Hz, 4'-$\boldsymbol{H}_2$), 1.86 – 1.79 (1H, m, 4-***H***H'), 1.74 – 1.68 (1H, m, 3-***H***H'), 1.65 – 1.57 (2H, m, 2'-$\boldsymbol{H}_2$), 1.59 – 1.54 (2H, m, 3-H***H***', 5-***H***H'), 1.54 – 1.49 (2H, m, 4-H***H***', 5-H***H***'), 1.49 – 1.43 (2H, m, 3'-$\boldsymbol{H}_2$); δ_C (176 MHz, $CDCl_3$) 138.9 (***C***-5'), 114.6 (***C***-6'), 99.0 (***C***-2), 67.5 (***C***-1'), 62.4 (***C***-6), 33.7 (***C***-4'), 30.9 (***C***-3), 29.4 (***C***-2'), 25.7(3) (***C***-3'), 25.6(5) (***C***-5), 19.8 (***C***-4); *m/z* (LCMS, ESI^+) 391 (M_2Na^+).

2-(3'-Bromopropoxy)tetrahydropyran[318] 214

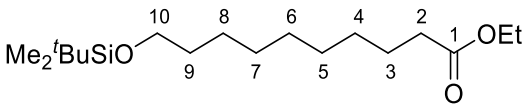


Procedure: A mixture of 3-bromopropan-1-ol (97%, 0.23 mL, 2.50 mmol), 3,4-dihydro-2*H*-pyran (97%, 0.35 mL, 3.75 mmol) and pyridinium *p*-toluenesulfonate (98%, 64 mg, 0.25 mmol) was stirred in DCM (10 mL) for 6 h. H_2O (8 mL) was added to quench the reaction before the mixture was passed through a phase separator. The organic extracts were concentrated *in vacuo* and purified on SiO_2 column (0% → 40% ether in hexanes) to afford the title compound (558 mg, quantitative) as a colourless clear oil.

R_f 0.43 (20% ether in hexanes); ν_{max} (ATR) 2941 (m), 2871 (m), 1441 (w), 1353 (m), 1201 (m), 1134 (m), 1076 (m), 1031 (s) cm^{-1}; δ_H (600 MHz, $CDCl_3$) 4.60 (1H, t, *J* = 3.5 Hz, 2-***H***), 3.90 – 3.84 (2H, m, 1'-***H***H', 6-***H***H'), 3.57 – 3.49 (4H, m, 1'-H***H***', 3'-$\boldsymbol{H}_2$, 6-H***H***'), 2.13 (2H, p, *J* = 6.5 Hz, 2'-$\boldsymbol{H}_2$), 1.87 – 1.76 (1H, m, 4-***H***H'), 1.76 – 1.67 (1H, m, 3-***H***H'), 1.64 – 1.47 (4H, m, 3-H***H***', 4-H***H***', 5-$\boldsymbol{H}_2$);

δ_C (151 MHz, $CDCl_3$) 99.0 (***C***-2), 65.1 (***C***-1'), 62.4 (***C***-6), 33.1 (***C***-2'), 30.8 (***C***-3'), 30.8 (***C***-3), 25.6 (***C***-5), 19.7 (***C***-4); *m/z* (GCMS, EI) 221 ($M(^{79}Br) - H^{\bullet}$), 223 ($M(^{81}Br) - H^{\bullet}$).

Ethyl 10-(*tert*-butyldimethylsilyloxy)decanoate 215



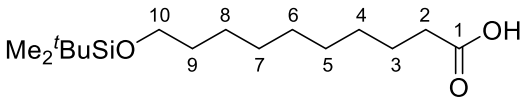
Procedure: A mixture of 10-hydroxydecanoic acid (403 mg, 2.14 mmol), concentrated H_2SO_4 (50 µL) and EtOH (10 mL) was stirred at reflux for 18 h. The reaction was then cooled, concentrated *in vacuo* and diluted with DCM (20 mL) prior to washing with 3 M $NaOH_{(aq.)}$ (3 × 20 mL). The organic fraction was then washed with brine (20 mL), dried over $MgSO_4$ and the volatiles removed under reduced pressure to furnish the crude ethyl 10-hydroxydecanoate (429 mg, 93%) as a pale yellow oil which was used without further purification.

To a mixture of ethyl 10-hydroxydecanoate (418 mg, 1.93 mmol), NEt_3 (99%, 0.33 mL, 2.32 mmol) and 4-dimethylaminopyridine (99%, 48 mg, 0.39 mmol) in DCM (2 mL) was added a solution of *tert*-butyldimethylsilyl chloride (97%, 315 mg, 2.03 mmol) in DCM (2 mL). The mixture was stirred for 4 h, after which TLC analysis revealed consumption of the reagent alcohol. The reaction was quenched by the addition of H_2O (5 mL) and the mixture passed through a phase separator. After removal of volatiles, the organic residue was purified *via* silica column chromatography (0% → 20% ether in hexanes) to afford the *title compound* (556 mg, 87%) as a clear colourless oil.

R_f 0.55 (20% ether in hexanes); ν_{max} (ATR) 2971 (w), 2928 (w), 2855 (w), 1737 (s), 1448 (w), 1366 (s), 1229 (s) cm^{-1}; δ_H (600 MHz, $CDCl_3$) 4.12 (2H, q, *J* = 7.0 Hz, $OC\boldsymbol{H}_2CH_3$), 3.59 (2H, t, *J* = 6.5 Hz, 10-$\boldsymbol{H}_2$), 2.28 (2H, t, *J* = 7.5 Hz, 2-$\boldsymbol{H}_2$), 1.61 (2H, p, *J* = 7.5 Hz, 3-$\boldsymbol{H}_2$), 1.52 – 1.46 (2H, m, 9-$\boldsymbol{H}_2$), 1.33 – 1.27 (10H, m, 8-$\boldsymbol{H}_2$, 4-$\boldsymbol{H}_2$, Al***H***), 1.25 (3H, t, *J* = 7.0 Hz, $OCH_2C\boldsymbol{H}_3$), 0.89 (9H, s, $OSi(C(C\boldsymbol{H}_3)_3)(CH_3)_2$), 0.04 (6H, s, $OSi(C(CH_3)_3)(C\boldsymbol{H}_3)_2$); δ_C (151 MHz, $CDCl_3$) 174.1 (***C***=O), 63.5 (***C***-10), 60.3 ($O\boldsymbol{C}H_2CH_3$), 34.6 (***C***-2), 33.0 (***C***-9), 29.6 (Al***C***), 29.5 (Al***C***), 29.4 (Al***C***), 29.3 (Al***C***), 26.1 ($OSi(C(\boldsymbol{C}H_3)_3)(CH_3)_2$), 25.9 (***C***-8), 25.1 (***C***-3), 18.5 ($OSi(\boldsymbol{C}(CH_3)_3)(CH_3)_2$), 14.42 ($OCH_2\boldsymbol{C}H_3$), −5.1

(OSi(C(CH$_3$)$_3$)(***C***H$_3$)$_2$); *m/z* (LCMS, ESI$^+$) 331 (MH$^+$); Accurate mass: Found MH$^+$, 331.2683: $C_{18}H_{39}O_3Si$ requires *M*, 331.2668.

10-(*tert*-Butyldimethylsilyloxy)decanoic acid[319] 216



Procedure: Ester **215** (515 mg, 1.56 mmol) and LiOH (74 mg, 3.12 mmol) were stirred in a mixture of THF (5 mL) and H_2O (5 mL) at 50 °C until TLC analysis displayed full saponification of the starting material. The mixture was then cooled in an ice bath and the pH adjusted to 4 using 1 M HCl, before being washed with EtOAc (3 × 10 mL). The combined organic extracts were washed with brine (20 mL), dried over $MgSO_4$ and concentrated *in vacuo*. SiO_2 column chromatography (0% → 40% ether in hexanes with 0.5% formic acid) of the crude mixture revealed the title compound (218 mg, 46%) as a clear colourless oil.

R_f 0.58 (20% ether in DCM with 1% formic acid); ν_{max} (ATR) 3017 (w), 2928 (w), 2856 (w), 1728 (s), 1455 (w), 1366 (s), 1229 (s), 1095 (m) cm^{-1}; δ_H (700 MHz, $CDCl_3$) 3.59 (2H, t, *J* = 6.5 Hz, 10-***H***$_2$), 2.33 (2H, t, *J* = 7.5 Hz, 2-***H***$_2$), 1.62 (2H, p, *J* = 7.5 Hz, 3-***H***$_2$), 1.50 (2H, p, *J* = 6.5 Hz, 9-***H***$_2$), 1.36 – 1.24 (10H, m, 8-***H***$_2$, 4-***H***$_2$, Al***H***), 0.89 (9H, s, OSi(C(C***H***$_3$)$_3$)(CH$_3$)$_2$), 0.04 (6H, s, OSi(C(CH$_3$)$_3$)(C***H***$_3$)$_2$); δ_C (176 MHz, $CDCl_3$) 180.4 (***C***=O), 63.5 (***C***-10), 34.3 (***C***-2), 33.0 (***C***-9), 29.6 (Al***C***), 29.5 (Al***C***), 29.3 (Al***C***), 29.2 (Al***C***), 26.1 (OSi(C(***C***H$_3$)$_3$)(CH$_3$)$_2$), 25.9 (Al***C***), 24.8 (***C***-3), 18.5 (OSi(***C***(CH$_3$)$_3$)(CH$_3$)$_2$), −5.1 (OSi(C(CH$_3$)$_3$)(***C***H$_3$)$_2$); *m/z* (LCMS, ESI$^-$) 301 (M − H$^+$).

9. Biological Experimental Details

9.1 General experimental details

MATERIALS: Biological grade materials, solvents, reagents and media components were purchased from commercial suppliers and used as provided. Plasmid construction and purification were carried out using the In-Fusion® HD cloning kit (Clontech) and plasmid miniprep kit (Biomiga) using supplied protocols. L-α-Phosphatidylinositol (bovine liver PI; predominant species 1-stearoyl-2-arachidonoyl-sn-glycero-3-phospho-1-myo-inositol sodium salt) was from Avanti Polar Lipids. NBD-C_6-ceramide and BODIPY® FL C_5-ceramide complexed to BSA were from Invitrogen. AG 4-X4 ion exchange resin was obtained from Bio-Rad. Reactions and media were prepared using high purity distilled, deionised water. FBS refers to heat-inactivated foetal bovine serum. Solutions of test compounds were made up in DMSO, unless otherwise stated.

INSTRUMENTS AND EQUIPMENT: 1.5 mL Protein LoBind tubes (Eppendorf) were used for handling microsomal material and metabolic labelling experiments. Media were filter-sterilised using a Corning® 1000 mL vacuum filter/storage bottle system with a 0.22 µm pore CA membrane. Centrifugation steps were carried out using Beckman Coulter® centrifuges and ultracentrifuges. Eppendorf tubes were centrifuged using a Sigma 1-14 microfuge. Disruption of yeast cells was performed using an IKA® Vortex Genius 3. Protein content and optical density (OD) were determined using a Boeco S-32 spectrophotometer. Eppendorf contents were dried using an Eppendorf Vacuum Concentrator 5301 from Brinkmann. Cells were counted using a Neubauer haemocytometer. 96-well plates used were Corning® Costar® cell culture plates 3596 (clear); Corning® Costar® cell culture plates 3595 (clear); Corning® V-bottom 3897 (clear); MultiScreen® Solvinert filter plates (Merck Millipore) and PerkinElmer OptiPlate-96 (black). 16-well tissue culture slides were supplied by Lab Tec®. Fluorescence quantification was carried out using Synergy H4 and FLx800 microplate readers with Gen5™ 1.08 data analysis software from Biotek. HPTLC silica plates were from Merck Millipore and imaged using a Fuji FLA−3000 plate reader with AIDA image analyser® software (version 3.52).

9.2 Solutions, buffers and media compositions

Buffer	Vol./Mass	Stock Solution
STE Buffer (50 mL)	1.25 mL	Tris-HCl (1 M, pH 7.4)
	12.5 mL	Sucrose (1 M)
	100 µL	EDTA (0.5 M)
	1 tablet	Complete® Protease Inhibitor Cocktail
	36.25 mL	Water
Storage Buffer (50 mL)	2.5 mL	Tris-HCl (1 M, pH 7.4)
	6.25 mL	80 % w/v Glycerol
	0.25 mL	$MgCl_2$ (1 M)
	1 tablet	Complete® Protease Inhibitor Cocktail
	41 mL	Water
Tris/EDTA/BSA Buffer	12.5 mL	Tris-HCl (1 M, pH 7.4)
(50 mL, pH 6.0)	2.5 mL	EDTA (0.5 M)
	750 mg	Fatty acid free BSA
	35 mL	Water
10 × TE Buffer (1 L)	100 mL	Tris-HCl (1 M, pH 7.4)
	20 mL	EDTA (0.5 M, pH 8.0)
	880 mL	Water
10 × TE/LiAc (50 mL)	5 mL	LiAc (1 M)
	5 mL	10 × TE Buffer
	40 mL	Water
10 × PEG/LiAc (50 mL)	5 mL	LiAc (1 M)
	5 mL	10× TE Buffer
	40 mL	50% w/v PEG 3350

Growth Media	Vol./Mass	Stock Solution
SD−W−L (1L)	20 g	Glucose
	± 15 g	Bacto-Agar (for solid media)
	1.93 g	Yeast Nitrogen Base (−Amino acids −$(NH_4)_2SO_4$)
	5 g	$(NH_4)_2SO_4$
	0.64 g	AA Drop-Out Supplement –W–L
	1000 mL	Water
SGR–W–L (± 5-FOA) (1L)	40 g	Galactose
	20 g	Raffinose pentahydrate
	± 15 g	Bacto-Agar (for solid media)
	1.93 g	Yeast Nitrogen Base (−Amino acids −$(NH_4)_2SO_4$)
	5 g	$(NH_4)_2SO_4$
	0.64 g	AA Drop-Out Supplement –W–L
	± 1 g	5-Fluoroorotic acid
	1000 mL	Water
SGR–W–L−URA (1L)	40 g	Galactose
	20 g	Raffinose pentahydrate

	± 15 g	Bacto-Agar (for solid media)
	1.93 g	Yeast Nitrogen Base (−Amino acids −$(NH_4)_2SO_4$)
	5 g	$(NH_4)_2SO_4$
	0.62 g	AA Drop-Out Supplement –W–L–URA
	1000 mL	Water
YPGR (1 L)	10 g	Yeast Extract
	20 g	Pepton
	40 g	Galactose
	20 g	Raffinose
	1000 mL	Water

9.3 Standard protocols

9.3.1 Complementation of *ΔAUR1 S. cerevisiae* mutant

The auxotrophic Δaur1 *S. cerevisiae* strain (α *ade⁻ lys⁻ leu⁻ AUR1Δ::TRP1* pRS316–Sc*AUR1*) was used for all transformation experiments. Yeast culture (5 mL in YPGR) at OD_{600} = 0.5 – 0.6 was transferred to a 50 mL tube and centrifuged (1,000 × g, RT, 5 min). The supernatant was discarded and the cells were re-suspended in sterile H_2O (5 mL). The cells were centrifuged (1,000 × g, RT, 5 min) and the supernatant was discarded. The cell pellet was the re-suspended in freshly prepared, sterile 1 × TE/LiAc buffer (1 mL) to form the competent yeast cells.

To the competent yeast cells (0.1 mL) was added the required plasmid (0.1 µg, pESC–LEU–IPC synthase) and Herring testes carrier DNA (10 µL, previously heated to 95 °C for 10 min). After mixing by vortex, freshly prepared sterile PEG/LiAc solution (0.6 mL) was added and the solution mixed by vortex prior to incubation at 30 °C for 30 min with shaking. DMSO (70 µL) was subsequently added and mixed by gentle inversion. The cells were subjected to a heat shock for 15 min at 42 °C and then chilled on ice for 2 min. Following centrifugation (14,000 rpm, RT, 5 s), the supernatant was removed and the cell pellet re-suspended in 1× TE buffer (0.5 mL) before being plated onto agar plates of permissive (SGR−W−L) and non-permissive media (SD−W−L). Counterselection was achieved through growth on agar plates of SGR–W–L+5-FOA medium.[261]

9.3.2 *ΔAUR1 S. cerevisiae* mutant culture

9.3.2.1 Agar plates

Stocks of the complemented yeast were stored at −80 °C in 80% glycerol and cultures were maintained in SGR−W−L agar medium. Plates were incubated at 30 °C and fully grown plate cultures were kept at 4 °C until use. Yeast colonies from these plates were used to inoculate liquid SGR−W−L medium.

9.3.2.2 Culture scale-up

Cultures were propagated in liquid SGR−W−L medium. Liquid culture (5 mL) was incubated at 30 °C until $OD_{600} \geq 0.8$. Fresh medium (500 mL) was added and the culture incubated at 30 °C until $OD_{600} \geq 0.8$. The culture was then diluted with fresh medium (5 L) and incubated at 30 °C until $OD_{600} = 0.5 – 0.6$.

9.3.3 Preparation of microsomal membrane fraction

9.3.3.1 Preparation of cell extract

Crude membranes from the mutant *S. cerevisiae* were prepared as described by Fischl *et al*.[320] Cells were harvested by centrifugation (4,000 × g, 4 °C, 10 min) and washed with non-sterile cold PBS buffer (3 × 20 mL). The cell pellet was weighed and re-suspended in STE buffer (1.5 mL per 1 g wet cell mass (WCM)). The yeast cells were disrupted using pre-chilled, acid washed glass beads (212–300 µm, 1.5 g per 1 g WCM), using a vortex mixer. Disruption involved 20 cycles of 1 min vortex mixing followed by 1 min resting on ice. Glass beads, unbroken cells and cell-wall debris were pelleted by centrifugation (1,500 × g, 4 °C, 15 min) and the cell extract (supernatant) removed and stored. The pellet was re-suspended in STE buffer (0.5 mL per 1 g WCM) and subjected to 10 further disruption cycles. Following centrifugation (1,500 × g, 4 °C, 15 min), the supernatant was removed and the cell extracts combined.

9.3.3.2 Preparation of crude microsomal membrane fraction

The microsomal membrane fraction, containing IPC synthase, was isolated from the cell extract by differential centrifugation. An initial centrifugation of the cell extract (27000 × g,

4 °C, 30 min) pelleted large organelles and cell debris. The supernatant was removed and re-centrifuged (150,000 × g, 4 °C, 90 min) to obtain a pellet enriched with microsomal membranes. The pellet was suspended in a minimal amount of storage buffer (generally 50 – 200 µL) and the protein content was determined according to Bradford's protocol.[321] The concentration was adjusted to 20 mg.mL^{-1} and 100 µL aliquots were stored in LoBind Eppendorf tubes at −80 °C.

9.3.3.3 Preparation of washed microsomal membranes

The following protocol was carried out under non-sterile conditions. The crude microsomal membranes were adjusted to a concentration of 10 mg.mL^{-1} using STE buffer and a 5% w/v CHAPS stock solution was diluted to 2.5% with STE buffer. Equal volumes of the membranes and 2.5% CHAPS solution were mixed and kept on ice for 1 h. The mixture was centrifuged (150,000 × g, 4 °C, 90 min) and the pellet re-suspended in storage buffer. The protein content was determined according to Bradford and the membranes were stored in LoBind Eppendorf tubes at −80 °C.[321]

9.3.3.4 Determination of protein activity in units (U)[209]

Microsome samples were normalized with respect to active enzyme content. Enzymatic activity was measured in enzyme units (U), where 1 U of enzyme is defined as that which converts 1 pmol of substrate per minute under the conditions described (*i.e*. 1 U = 1 pmol(product).min^{-1}).

A stock solution of NBD-C_6-ceramide **34** at a concentration of 100 µM was used to create a standard curve ranging from 0.2 pmol to 80 pmol. The volumes were adjusted to 200 µL with 1 M potassium formate in MeOH and the fluorescence measured (Ex460/Em540). Samples of the washed microsomal membranes were incubated with NBD-C_6-ceramide **34** and PI under assay conditions (see Section 8.4.2) and the product fluorescence measured. Correlation with the standard curve allowed the activity of the microsome preparation to be calculated in U.µL^{-1}. The membranes were adjusted to 1.5 U.µL^{-1} with storage buffer and stored in LoBind Eppendorf tubes at −80 °C.

9.3.4 *Leishmania* culture

Leishmania major (MHOM/IL/81/Friedlin; FV1 strain (WT and *spt2*$^-$) and MHOM/SA/85/JISH118) promastigotes were maintained at 26 °C in Schneider's insect medium (pH 7), supplemented with 15% heat-inactivated foetal bovine serum.

9.4 Assay protocols

9.4.1 HPTLC NBD-C_6-ceramide fluorescence assay[322]

10 mM PI in $CHCl_3$ (1 µL) was dried into a LoBind Eppendorf tube using a vacuum concentrator (Eppendorf Concentrator 5301). To the dried PI, Tris/EDTA/BSA buffer (20 µL) was added and the solution mixed by vortex. The volume was adjusted to 48 µL with distilled H_2O, followed by the addition of test compound (0.5 µL) and 100 µM NBD-C_6-ceramide **34** in DMSO (1 µL). The reaction was started by addition of microsomal membranes (1.5 U.µL^{-1}, 0.5 µL), and the mixture incubated at 30 °C for 25 min. After quenching with $CHCl_3$: MeOH : H_2O (10 : 10 : 3, 150 µL), the mixture was centrifuged (14,400 × g, RT, 5 min). 50 µL of the organic layer was loaded onto a HTPLC plate, and the lipid components separated using the solvent system $CHCl_3$: MeOH : 0.25% $KCl_{(aq.)}$ (55 : 45 : 10). The R_f values for the substrate NBD-C_6-ceramide **34** and the product NBD-C_6-IPC **35** were 0.96 and 0.57 respectively. Product quantification was carried out using a fluorescence plate reader (Ex473/Em520).

9.4.2 96-well plate NBD-C_6-ceramide fluorescence assay[322]

In a V-bottom 96-well plate, CHAPS-washed membranes (0.6 U) were used in a reaction mix containing 100 µM PI, 5 µM NBD-C_6-ceramide **34**, 50 mM PO_4^- and 600 µM CHAPS. Per well, 10 mM PI in $CHCl_3$ (0.4 µL) was dried using a vacuum concentrator. To the dried PI was added 200 µM NBD-C_6-ceramide **34** in DMSO (1 µL), H_2O (1.1 µL), 3 mM $CHAPS_{(aq.)}$ (8 µL) and 71.4 mM phosphate buffer (28 µL). Test compounds in DMSO (0.4 µL) were added, and mixed thoroughly. The reaction was initiated by the addition of microsomal material (1.5 U.µL^{-1}, 0.4 µL) diluted with storage buffer (1.1 µL). The reaction was conducted with shaking at 30 °C for 25 min, prior to quenching by the addition of 200 µL of MeOH.

Separation of the reaction product, NBD-C_6-IPC **35**, from the substrate NBD-C_6-ceramide **34** was achieved *via* anion exchange chromatography. 20% w/v AG4-X4 resin in EtOH (200 µL) was dispensed into 96-well filter plates and the plate centrifuged (2,450 × g,

RT, 30 s). Formic acid (50 µL) was added to each well and, after 5 min, excess acid was removed by centrifugation (2,450 × g, RT, 30 s). The protonated resin was then washed with H_2O (100 µL) and centrifuged (2,450 × g, RT, 30 s). The quenched reaction mixture (200 µL) was loaded onto the prepared resin and centrifuged (2,450 × g, RT, 30 s). Excess material was removed by washing with MeOH (5 × 200 µL) and subsequent centrifugation (2,450 × g, RT, 30 s). The product IPC **35** was eluted into a black plate by washing with 1 M potassium formate in MeOH (4 × 50 µL) followed by centrifugation (2,450 × g, RT, 30 s). Assays were carried out in triplicate, with quantification carried out using a fluorescence plate reader (Ex460/Em540). IC_{50} values were calculated using sigmoidal regression analysis (GraphPad Prism).

9.4.3 Metabolic labelling assay

Cultured *L. major spt2*$^-$ mutant promastigotes were centrifuged (1,500 × g, RT, 10 min) and the pellet washed three times with Schneider's insect medium (pH 7, no FBS). The pellet was subsequently re-suspended in Schneider's insect medium (pH 7, no FBS) to a parasite concentration of 1×10^7 mL^{-1} and dispensed into Eppendorf tubes (250 µL). Following incubation at 26 °C for 1 h, the test compounds were added (1 µL) and the tubes incubated at 26 °C for 18 h. The reaction was initiated by the addition of 0.5 mM BODIPY FL C_5-ceramide complexed to BSA (1.25 µL). Following further incubation at 26 °C for 1 h, the tubes were centrifuged (12,500 × g, RT, 5 min) and the pellets washed twice with PBS (250 µL). The pellets were re-suspended in $CHCl_3$: MeOH : H_2O (10 : 10 : 3, 200 µL), followed by sonication in a water bath for 15 min. Water (25 µL) was added before centrifugation (14,400 × g, RT, 10 s) of the cell lysate. 50 µL of the organic layer was loaded onto a HTPLC plate, and lipid components separated using the solvent system $CHCl_3$: MeOH : 0.25% $KCl_{(aq.)}$ (55 : 45 : 10). The R_f values for the substrate BODIPY FL C_5-ceramide and the product BODIPY FL C_5-IPC were 0.89 and 0.47 respectively. Product quantification was carried out using a fluorescence plate reader (Ex473/Em520). IC_{50} values were calculated using sigmoidal regression analysis (GraphPad Prism).

9.4.4 *L. major* promastigote assay[214]

L. major promastigotes (100 µL at 4×10^5 mL^{-1}) were incubated in sterile 96-well plates with compounds in triplicate (amphotericin B was used as a positive control, and untreated parasites with DMSO as a negative control) at 26 °C for 24 h. AlamarBlue® (10 µL) was then added and the plate incubated at 26 °C for 4 h prior to measurement using a

fluorescence plate reader (Ex540/Em600). ED_{50} values were calculated using sigmoidal regression analysis (GraphPad Prism).

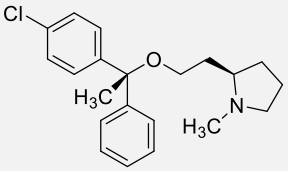
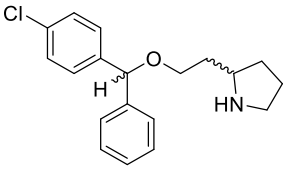
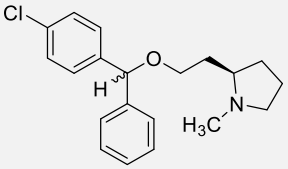
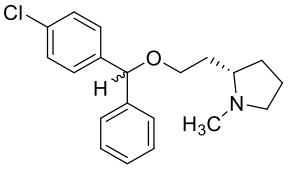
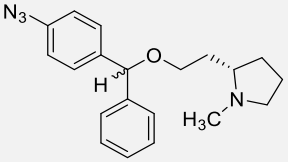
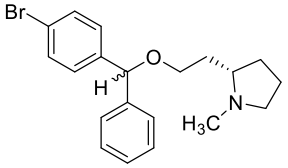
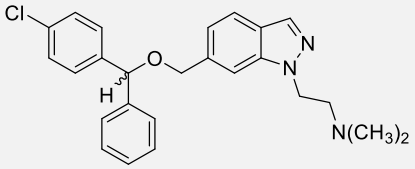
9.4.5 *L. major* intramacrophage amastigote assay[323]

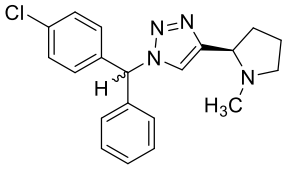
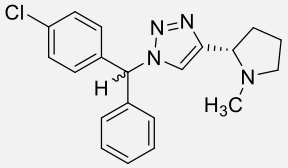
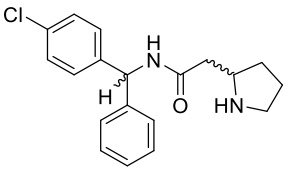
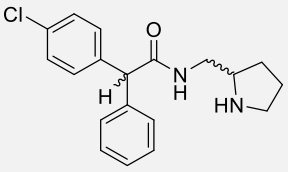
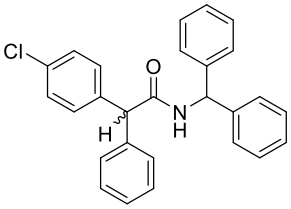
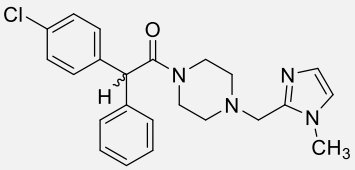
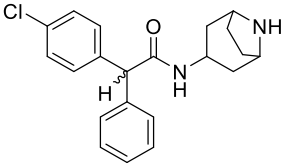
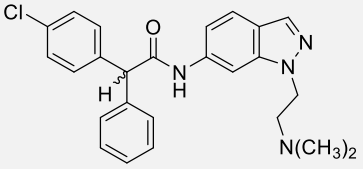
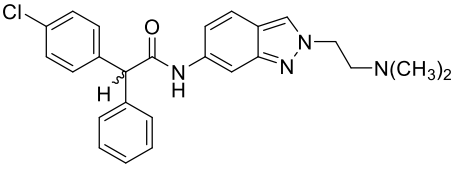
Primary peritoneal mouse (CD1) macrophages were diluted in RPMI 1640 medium (supplemented with 200 mM L-glutamine, 16.5 mM $NaHCO_3$ and 10% FBS) to a concentration of 4×10^5 mL^{-1}. An aliquot of 100 µL of culture was added to 16-well tissue culture slides and incubated for 24 h at 37 °C and 5% CO_2. Then, 100 µL of *L. major* (JISH118) promastigotes (in supplemented RPMI 1640 medium) were added at a ratio of seven promastigotes to one macrophage and the cells were incubated at 34 °C and 5% CO_2. After 24 h, extracellular parasites were removed and the infected cells were gently washed with cold medium (lacking FBS). Serial dilutions of the test compounds in medium (100 µL) were added and the slides were incubated at 34 °C and 5% CO_2 for 5 days (including replacement with fresh drug-containing medium after 72 h). Amphotericin B and miltefosine were used as controls. Parasite growth was microscopically assessed after methanol fixation and staining with a 10% Giemsa solution. The level of infection was evaluated by counting the percentage of infected macrophages per well. Assays were performed in quadruplicate, with results expressed as the percentage reduction of infected cells (compared with untreated control wells). ED_{50} values were calculated using sigmoidal regression analysis (GraphPad Prism).

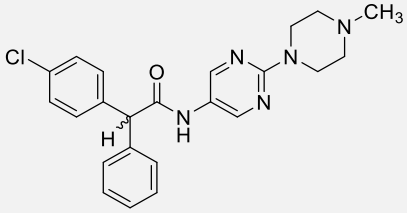
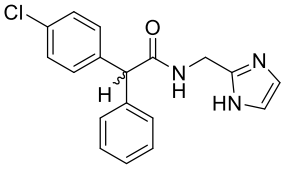
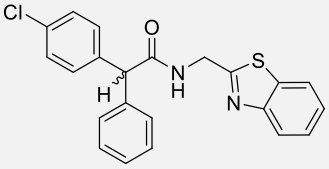
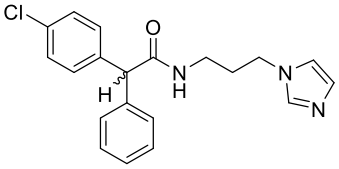
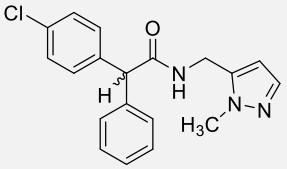
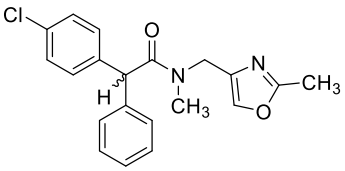
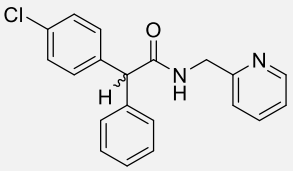
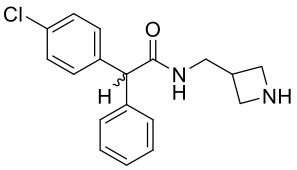
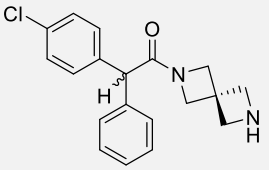
9.4.6 Macrophage cytotoxicity assay[324]

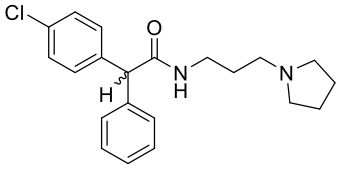
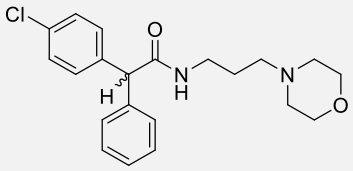
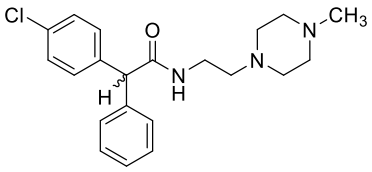
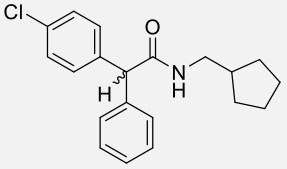
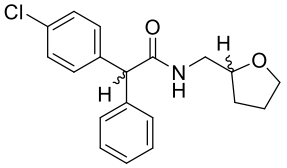
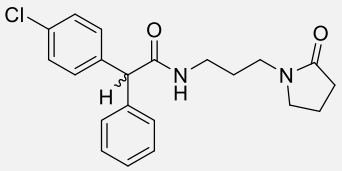
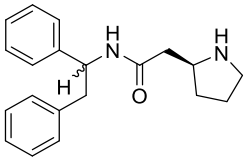
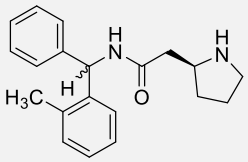
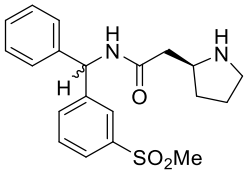
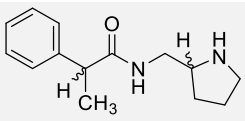
Primary peritoneal mouse (CD1) macrophages in RPMI 1640 medium (supplemented with 200 mM L-glutamine, 16.5 mM $NaHCO_3$ and 10% FBS) (2×10^6 mL^{-1}, 200 µL.$well^{-1}$) were seeded in sterile 96-well plates and incubated for 24 h at 37 °C and 5% CO_2. Following removal of media, serial dilutions of the test compounds in fresh medium (200 µL) were added and the plate was incubated for 24 h at 37 °C and 5% CO_2. Aliquots of AlamarBlue® (20 µL) were then added and the plate was incubated at 37 °C and 5% CO_2 for 4 h. Cell-viability measurement was carried out using a fluorescence plate reader (Ex540/Em600). Podophyllotoxin was used as a reference compound. ED_{50} values were calculated using sigmoidal regression analysis (GraphPad Prism).

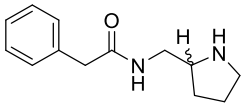
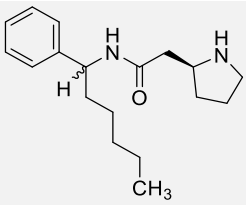
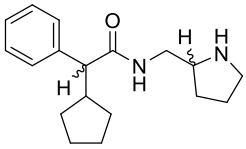
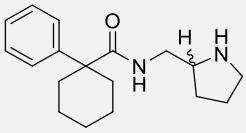
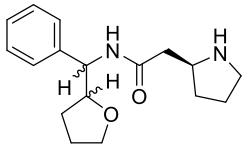
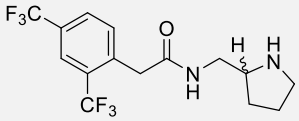
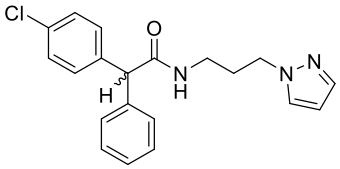
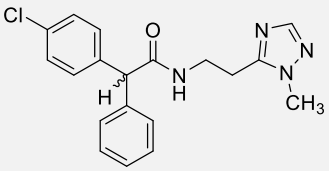
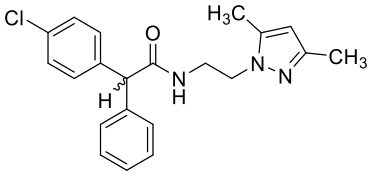
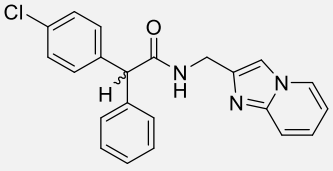
Appendix A: Clemastine Analogue Screening Data

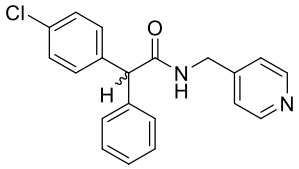
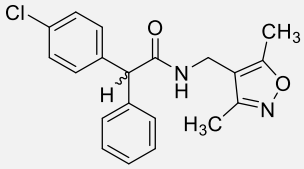
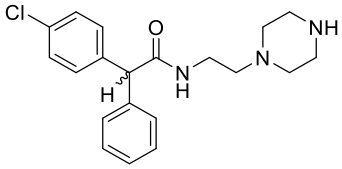
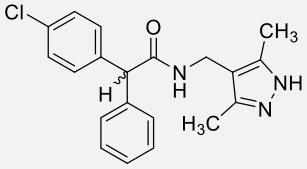
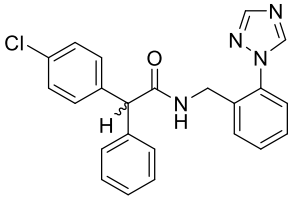
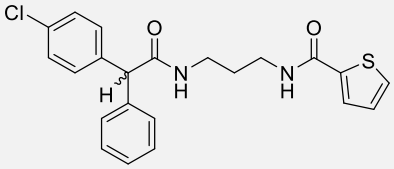
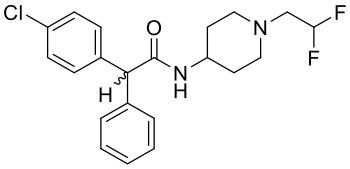
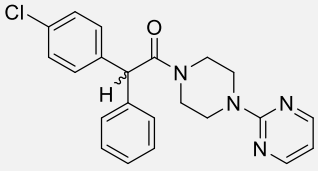
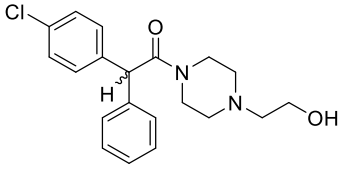
N°	Structure	*Lmj*IPCS inhibition % at 20 µM ± SD	*Lmj*IPCS IC_{50} (µM) (95% CI)	*L. major* promastigote ED_{50} (µM) (95% CI)
41	 Clemastine	82.6 ± 9.3	2.87 (2.25 – 3.66)	0.47 (0.37 – 0.59)
77		78.1 ± 12.5	6.26 (5.60 – 7.00)	4.10 (3.35 – 5.01)
78B		78.1 ± 9.7	4.13 (3.72 – 4.59)	0.78 (0.63 – 0.96)
78A		81.2 ± 10.6	4.45 (4.01 – 4.94)	1.69 (1.23 – 2.32)
162		–	3.66 (3.24 – 4.14)	2.85 (2.28 – 3.56)
166		–	3.21 (2.38 – 4.33)	1.36 (1.08 – 1.71)
146		–	4.72 (3.90 – 5.72)	>5

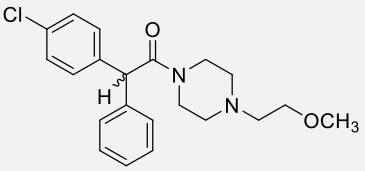
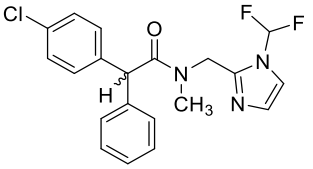
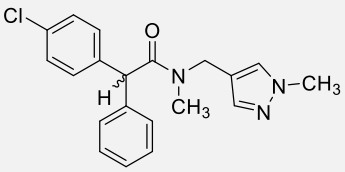
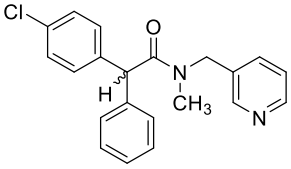
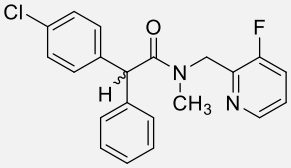
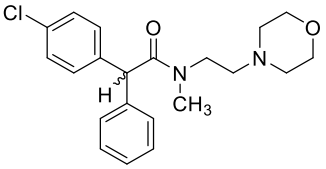
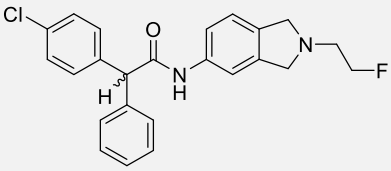
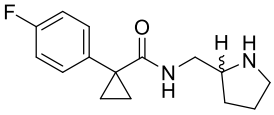
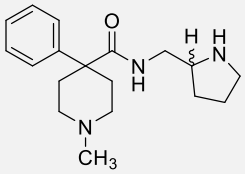
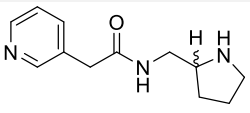
83B		71.0 ± 9.7	8.49 (7.67 – 9.39)	>33
83A		60.6 ± 9.2	14.52 (12.39 – 17.02)	>33
98		82.7 ± 1.3	6.95 (6.15 – 7.85)	>33
99		81.2 ± 1.3	6.90 (6.10 – 7.80)	>33
110		4.7 ± 2.5	–	–
111		8.6 ± 2.1	–	–
117		89.2 ± 2.6	5.40 (3.99 – 7.30)	–
118		90.2 ± 2.4	2.41 (1.84 – 3.16)	>10
119		90.2 ± 1.7	4.37 (3.56 – 5.36)	–

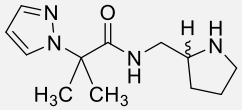
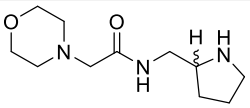
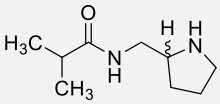
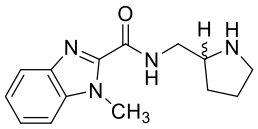
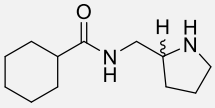
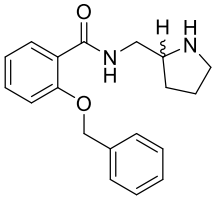
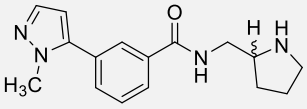
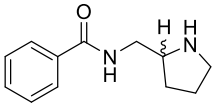
120		89.6 ± 2.2	6.11 (4.83 – 7.72)	–
121		53.4 ± 8.5	23.89 (20.84 – 27.39)	–
122		79.4 ± 9.0	36.21 (30.06 – 43.61)	–
123		28.3 ± 5.3	–	–
124		2.5 ± 7.8	–	–
125		0.6 ± 1.8	–	–
126		2.7 ± 1.7	–	–
127		86.0 ± 2.7	13.09 (11.43 – 15.00)	–
128		77.9 ± 5.1	11.38 (9.03 – 14.34)	–

129		76.1 ± 8.5	20.49 (18.30 – 22.94)	–
130		35.8 ± 3.9	–	–
131		16.3 ± 3.8	–	–
132		−5.4 ± 2.3	–	–
133		2.9 ± 1.4	–	–
134		−0.3 ± 0.8	–	–
135		44.4 ± 4.9	24.86 (22.22 – 27.82)	–
136		89.4 ± 4.1	18.94 (16.83 – 21.32)	–
137		11.3 ± 7.4	–	–
138		−1.1 ± 2.5	–	–

139		1.7 ± 3.4	–	–
140		81.9 ± 2.8	12.67 (11.12 – 14.44)	–
141		46.4 ± 5.8	28.31 (25.50 – 31.34)	–
142		24.7 ± 7.5	–	–
143		7.3 ± 1.9	–	–
144		57.2 ± 10.9	16.51 (12.80 – 21.29)	–
167		7.5 ± 7.3	–	–
168		2.4 ±1.7	–	–
169		7.3 ± 6.5	–	–
170		24.4 ± 3.3	–	–

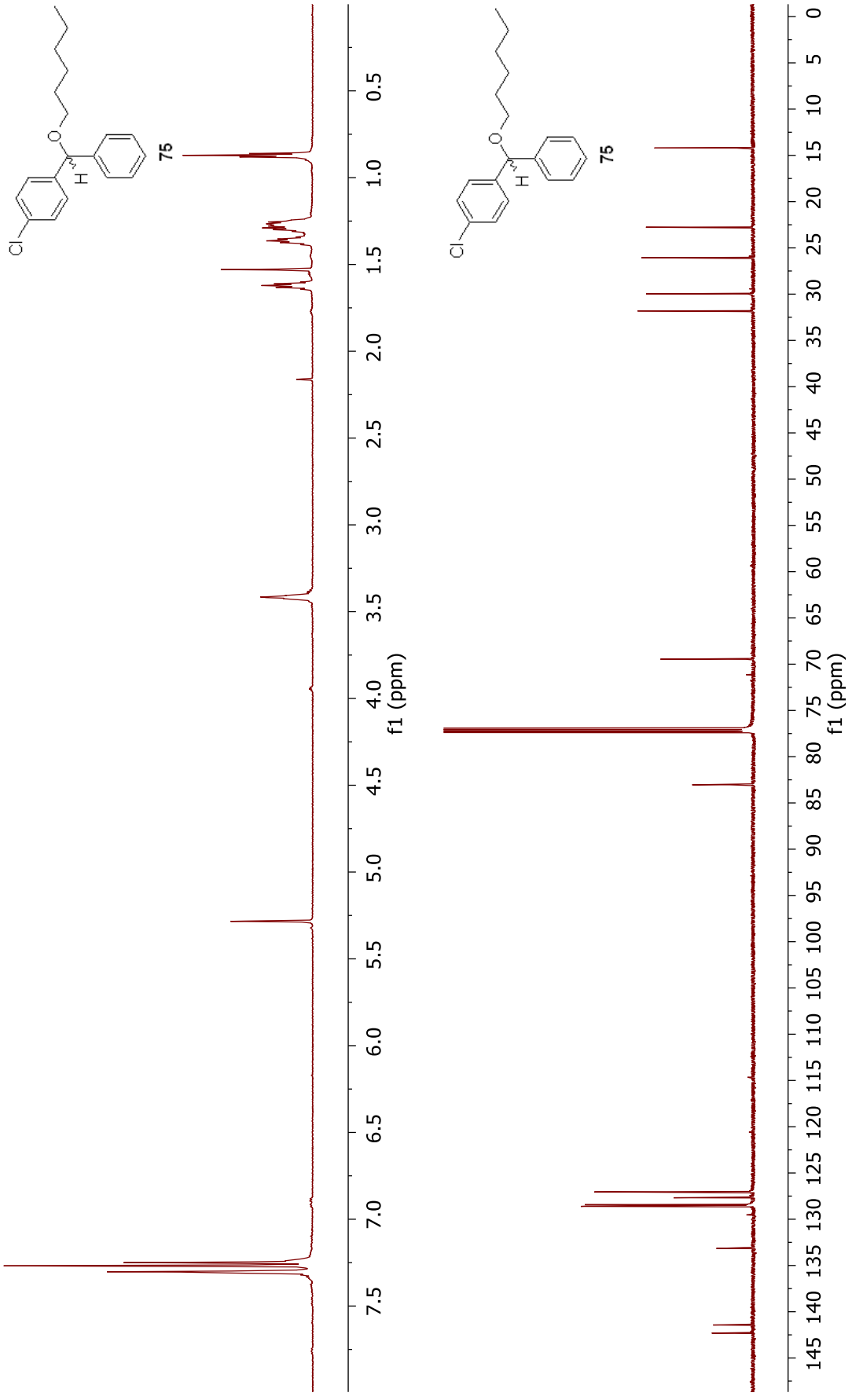
171		0.5 ± 2.1	–	–
172		12.0 ± 3.1	–	–
173		27.5 ± 8.3	–	–
174		4.3 ± 1.8	–	–
175		4.3 ± 3.1	–	–
176		9.0 ± 2.1	–	–
177		3.5 ± 3.1	–	–
178		−1.7 ± 5.3	–	–
179		16.0 ± 0.6	–	–

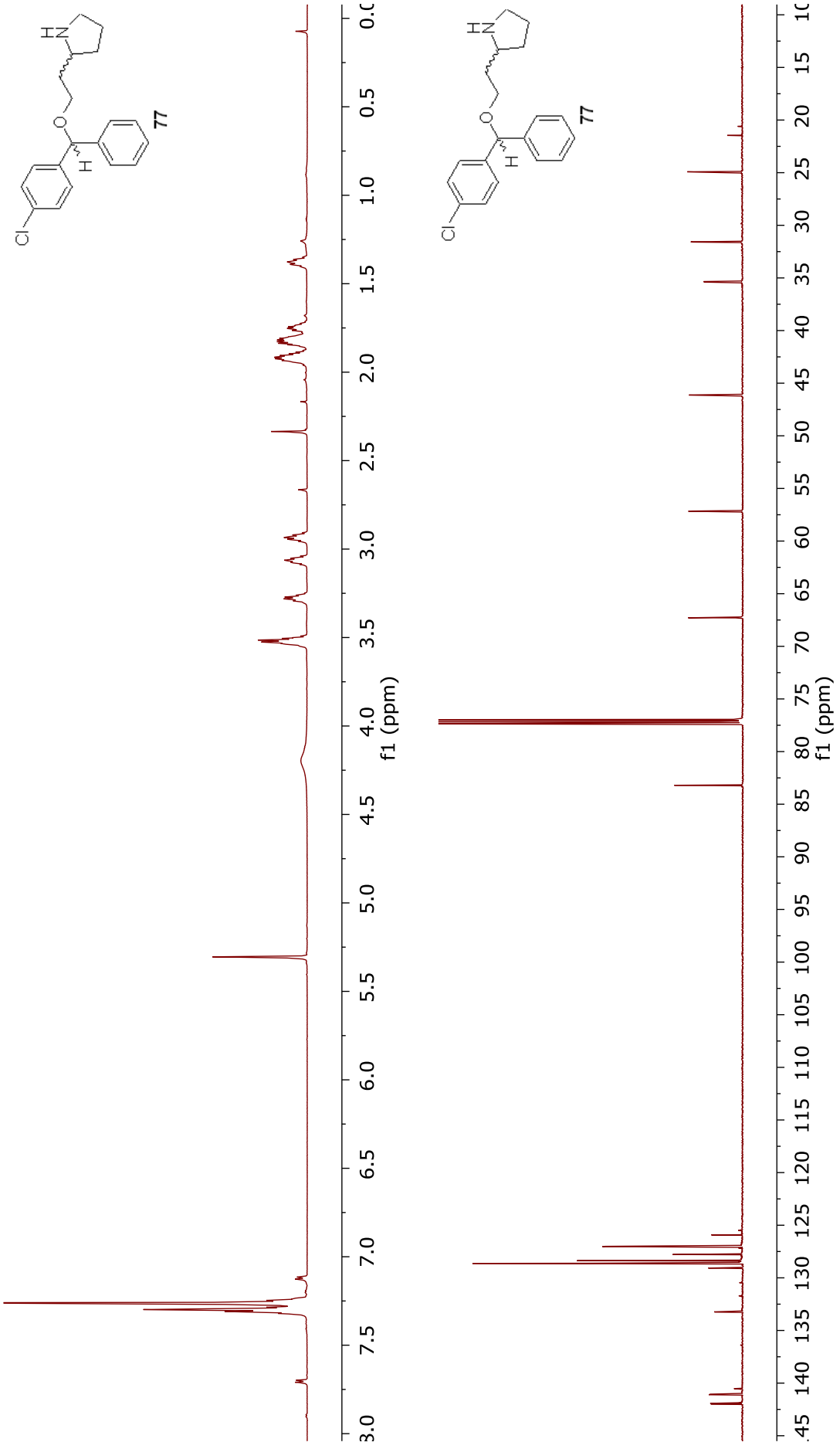
180		9.3 ± 1.8	–	–
181		8.5 ± 1.2	–	–
182		−3.2 ± 1.7	–	–
183		8.0 ± 1.2	–	–
184		5.1 ± 1.5	–	–
185		5.5 ± 3.2	–	–
186		59.8 ± 29.9	17.07 (13.48 – 21.62)	–
187		0.4 ± 5.3	–	–
188		26.5 ± 12.1	–	–
189		−1.8 ± 6.4	–	–

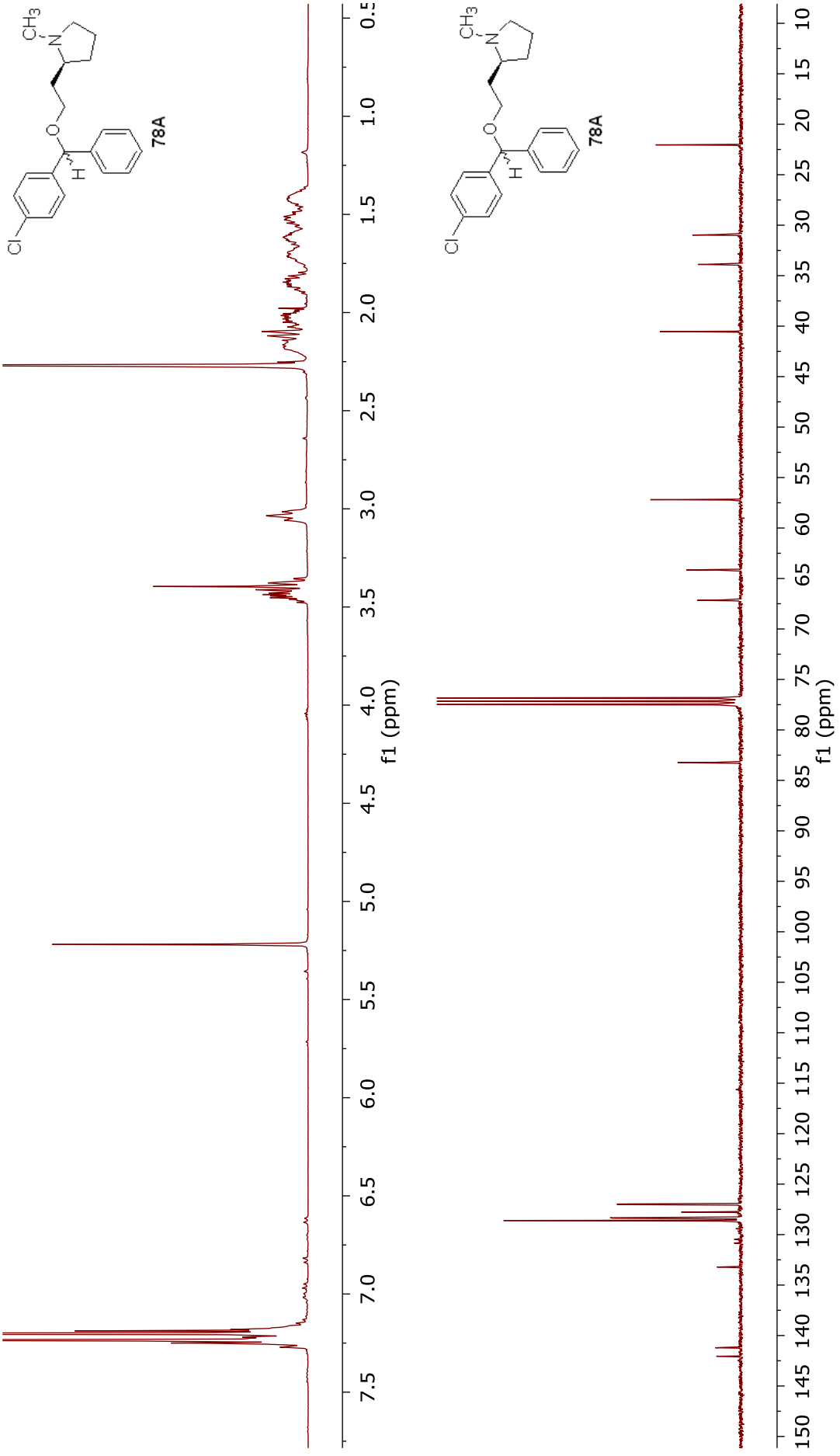
190		−1.5 ± 4.2	–	–
191		−1.1 ± 8.2	–	–
192		−4.9 ± 3.6	–	–
193		0.9 ± 5.3	–	–
194		−1.3 ± 3.7	–	–
195		40.6 ± 15.7	–	–
196		−2.1 ± 2.7	–	–
197		−0.7 ± 2.4	–	–

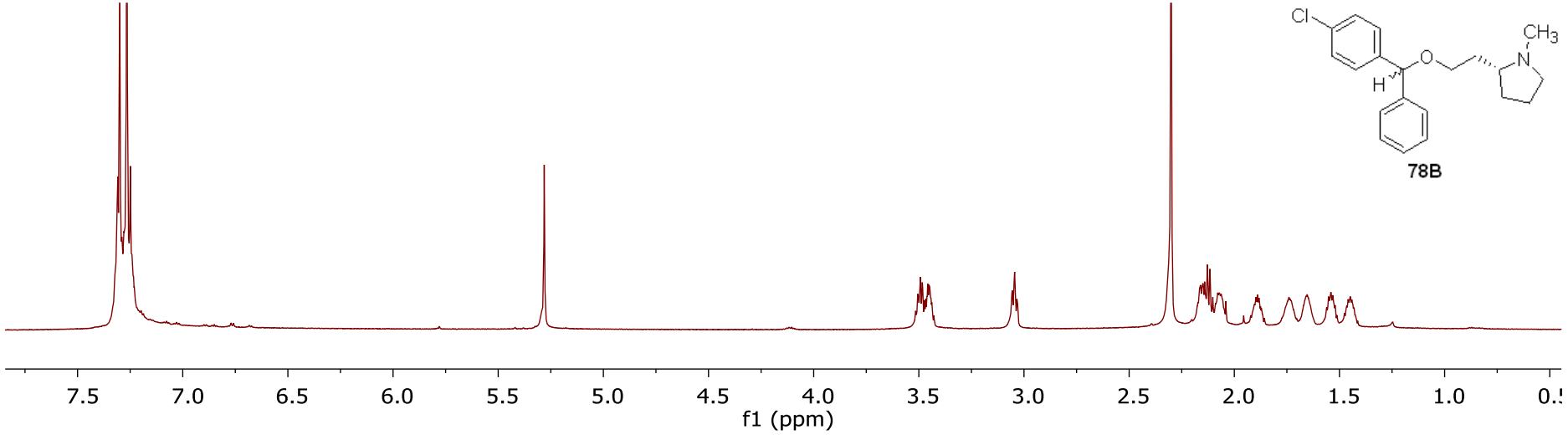
– Value not determined

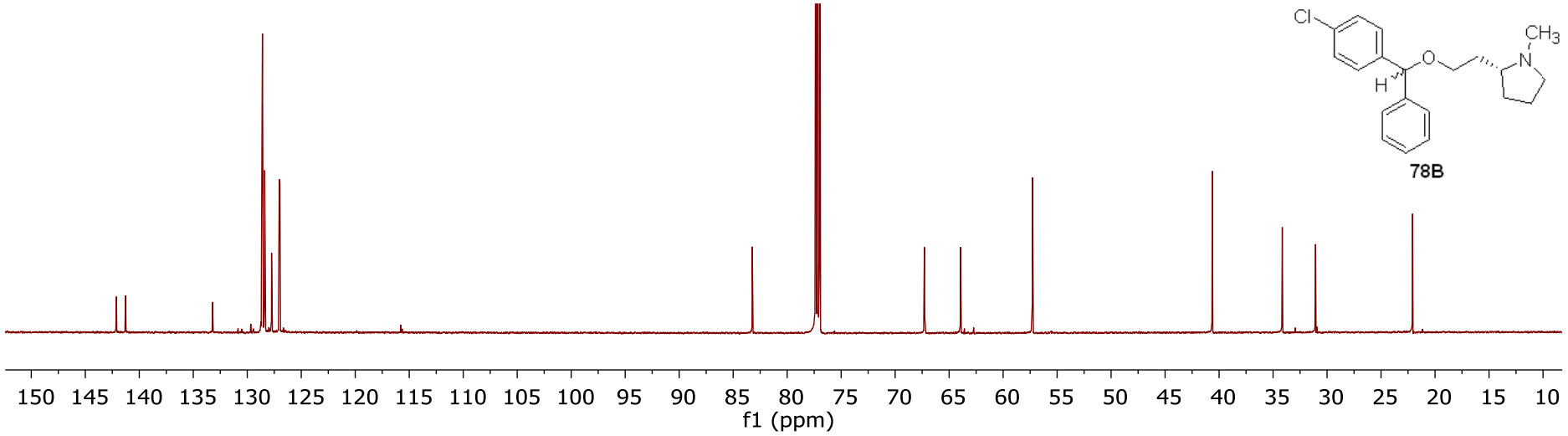
Appendix B: NMR Spectra

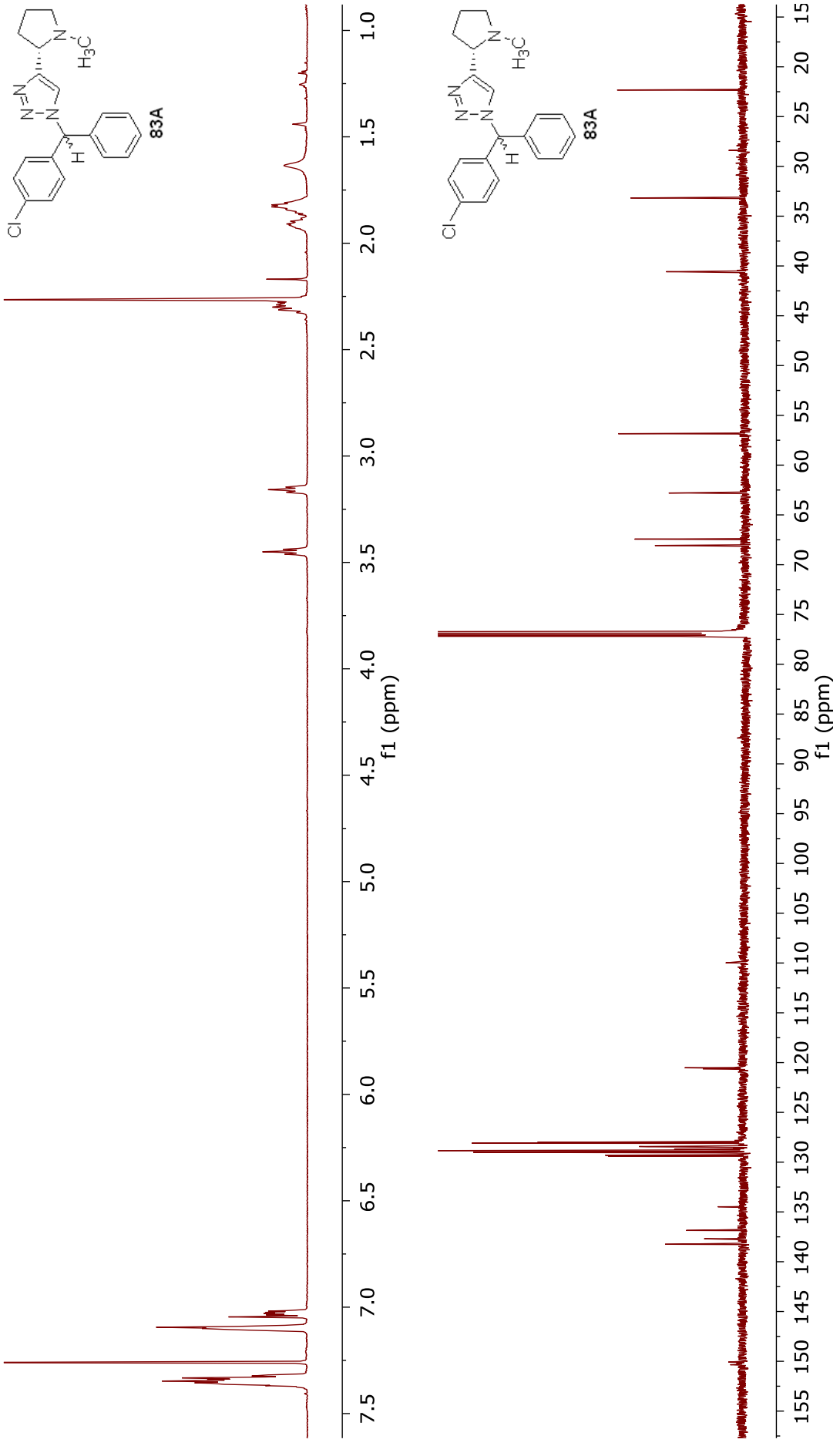


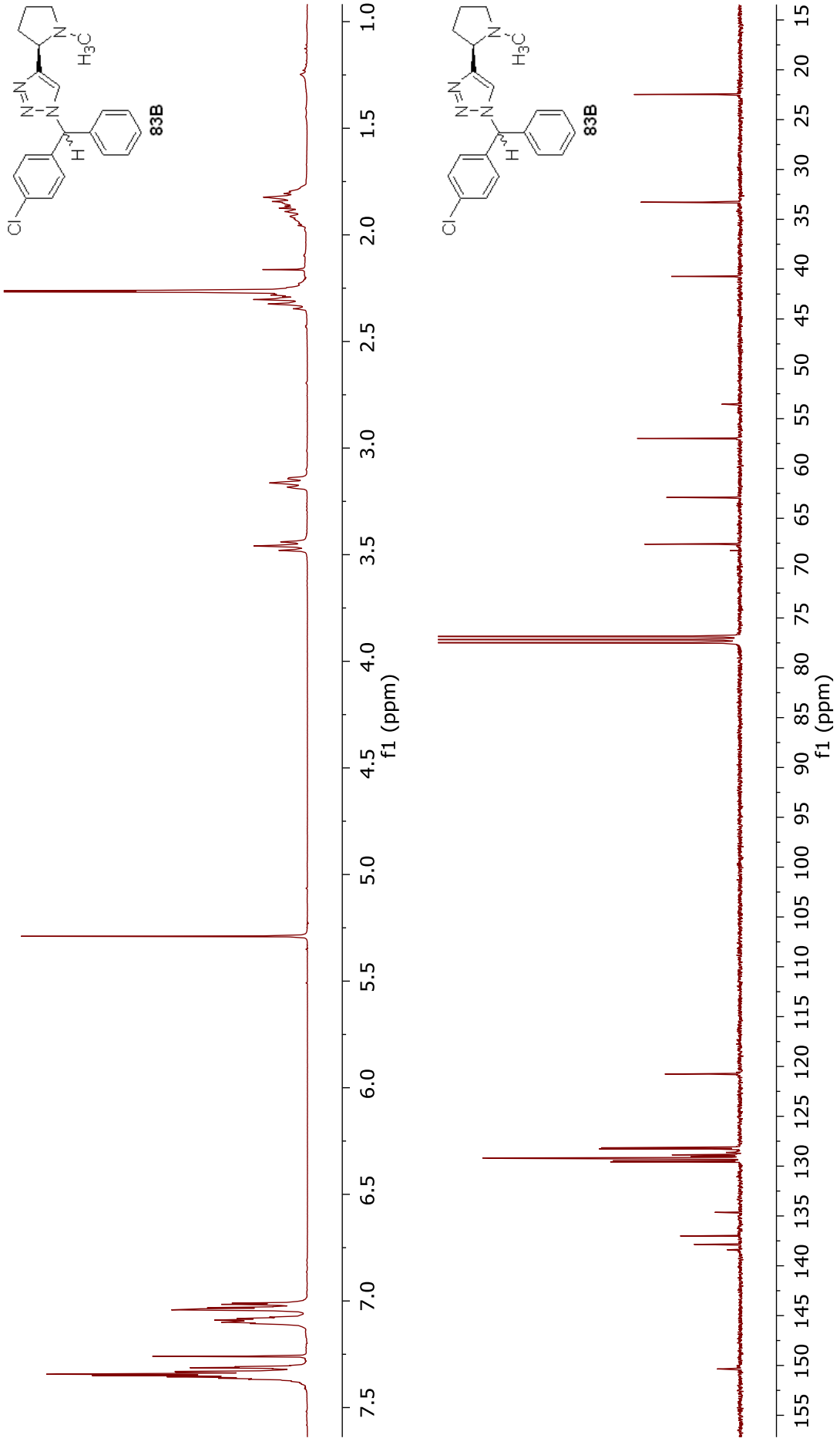

77
f1 (ppm)
77
f1 (ppm)

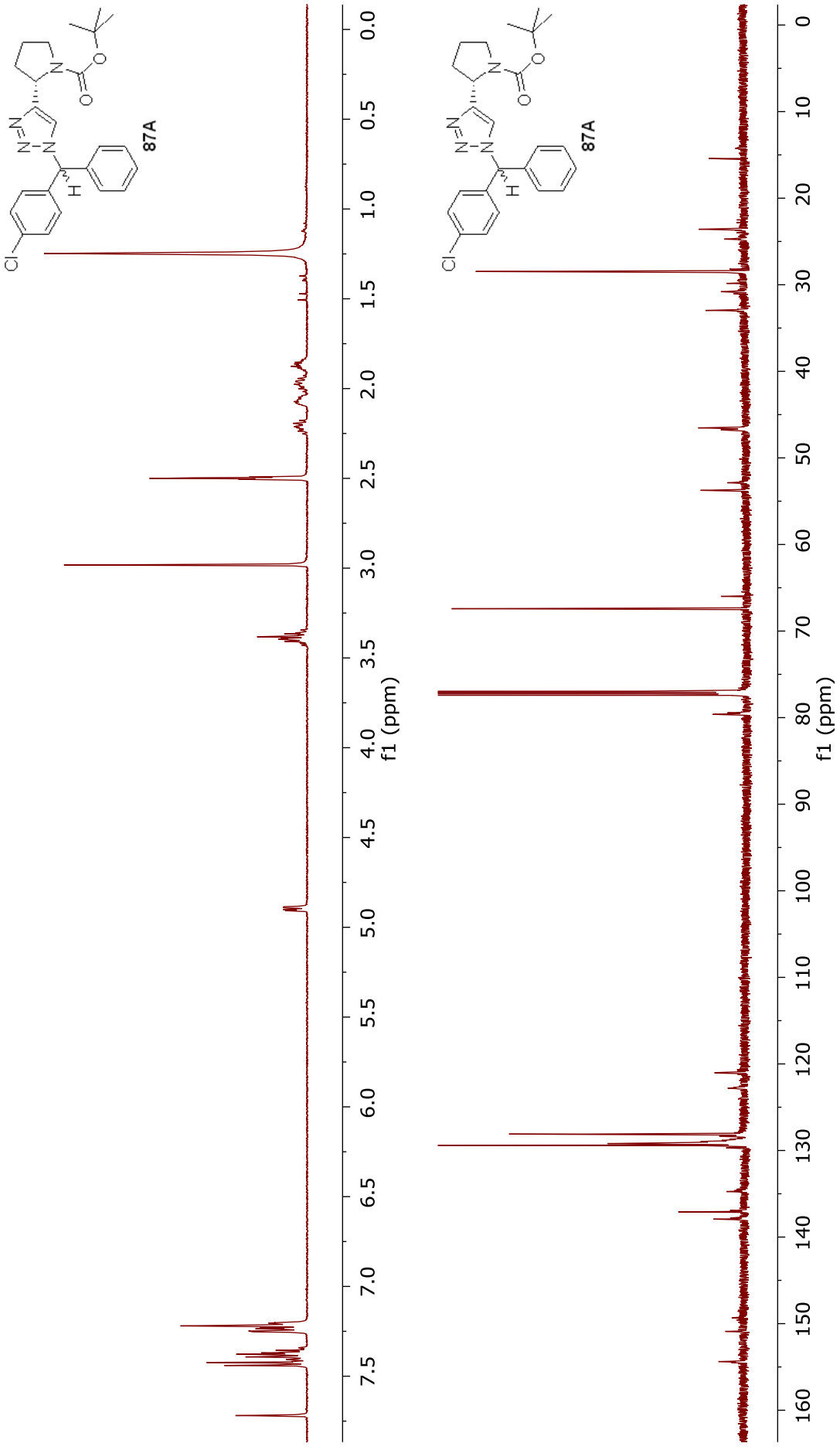

78A
f1 (ppm)
78A
f1 (ppm)

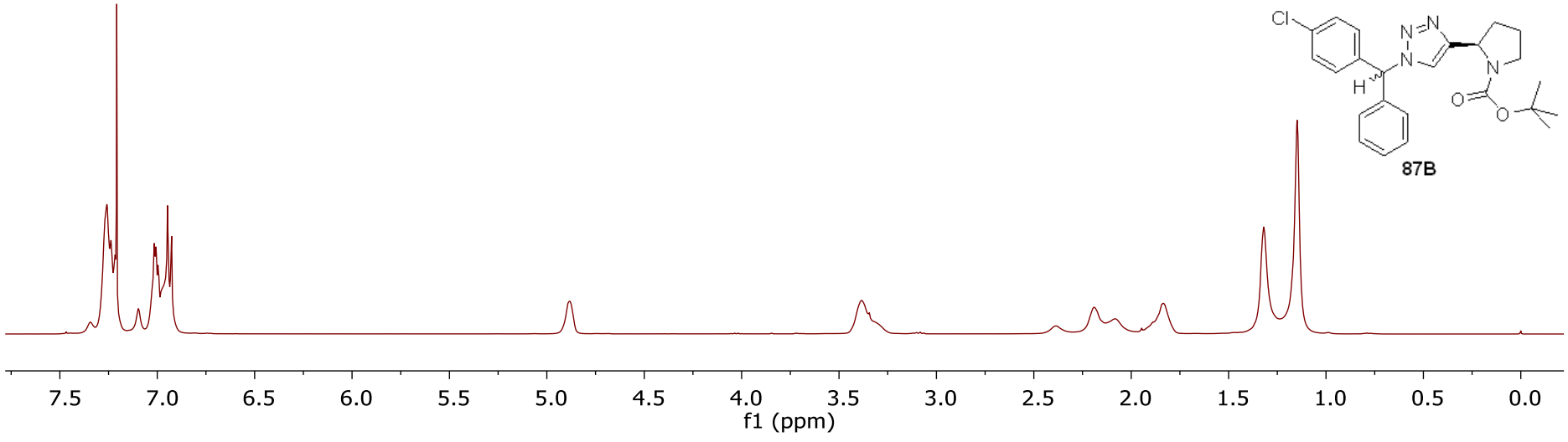

Cl
CH3
O
N
H
78B
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)

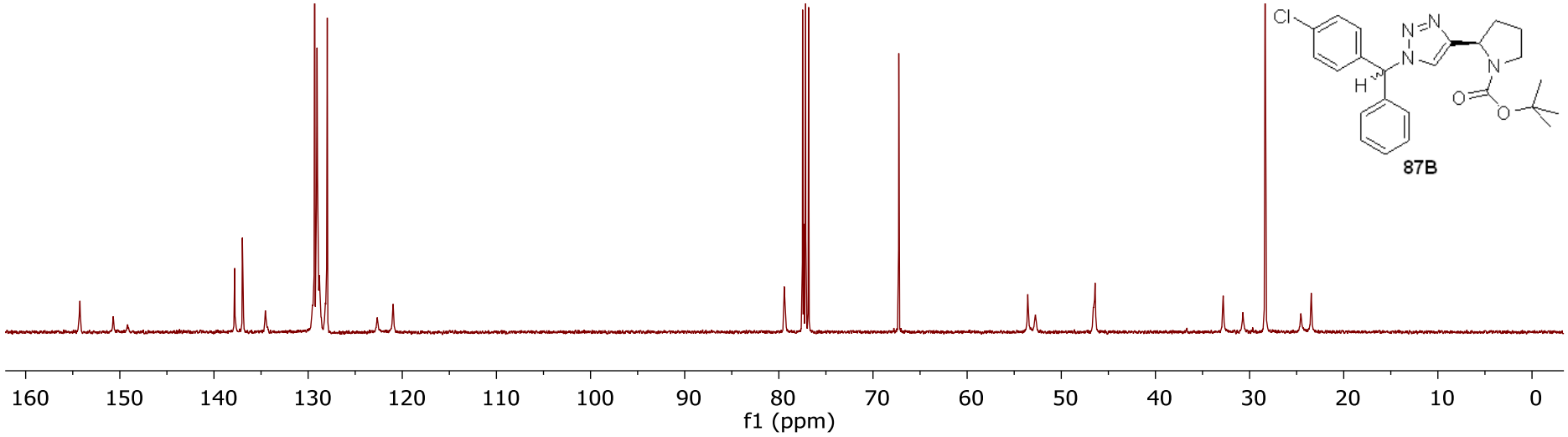

Cl
CH3
O
N
H
78B
150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10
f1 (ppm)

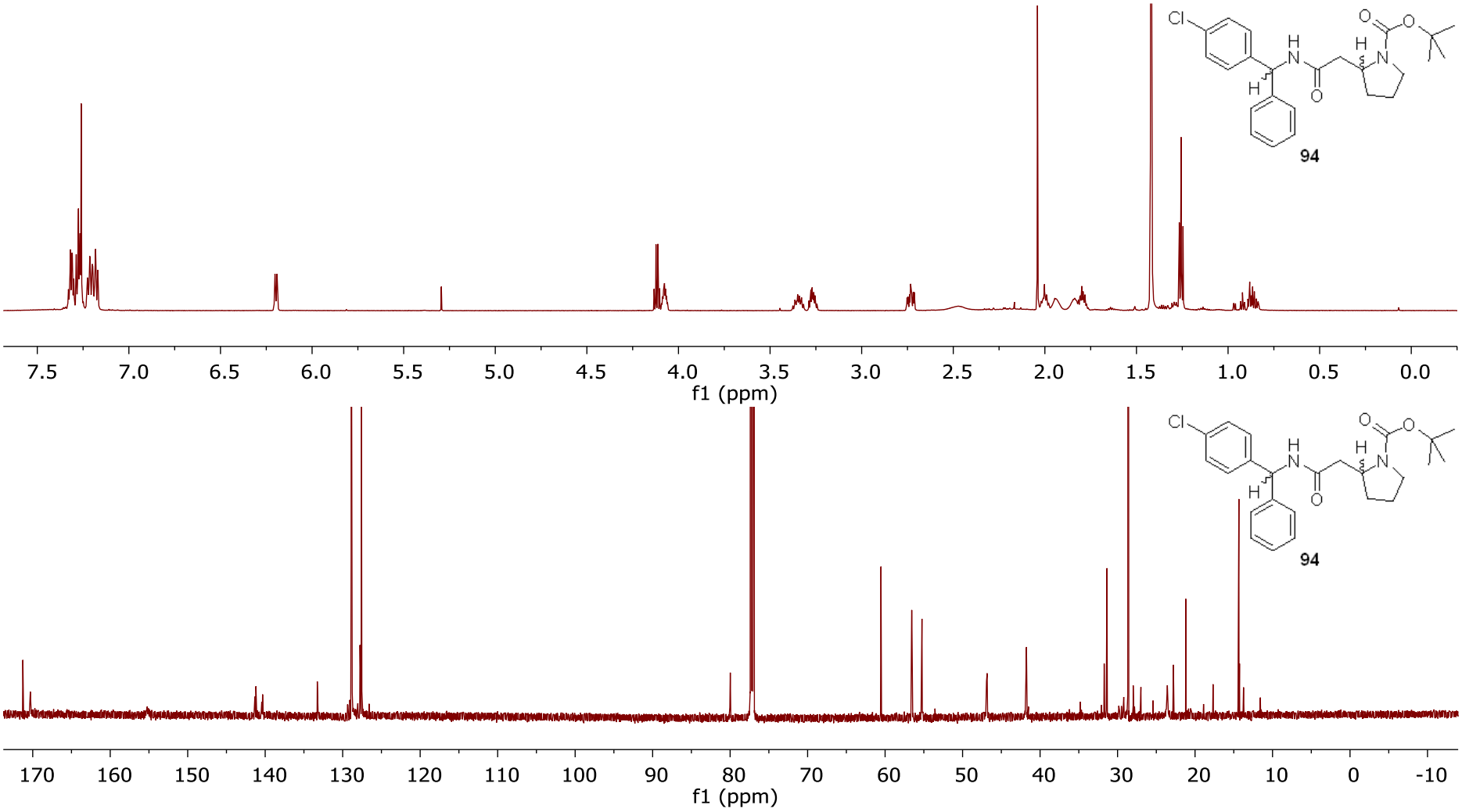

83A
f1 (ppm)
83A
f1 (ppm)


83B
f1 (ppm)
83B
f1 (ppm)

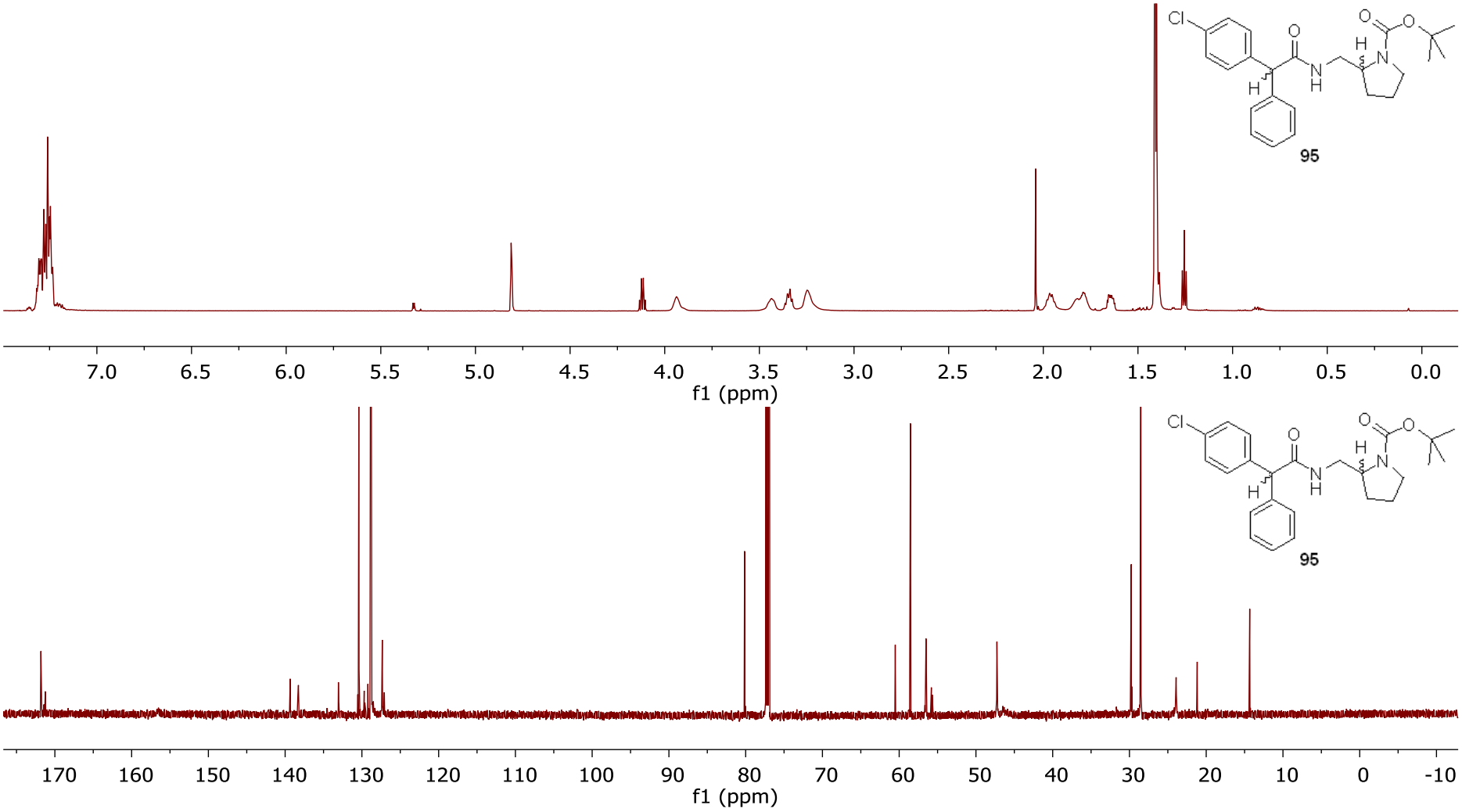

87A
f1 (ppm)
87A
f1 (ppm)


Cl
N
N
N
H
O
O
N
87B
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

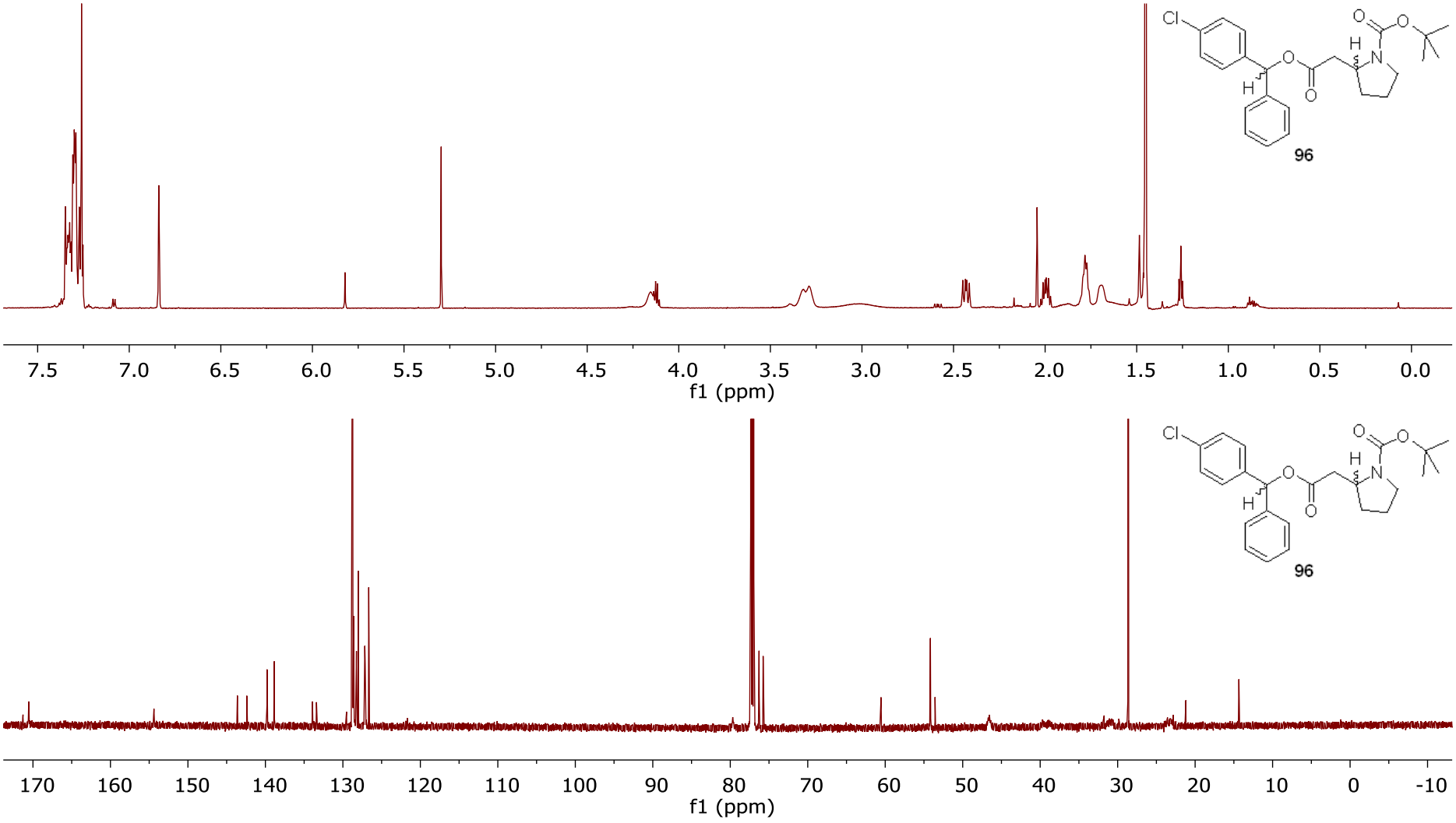

Cl
N
N
N
H
O
O
N
87B
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)



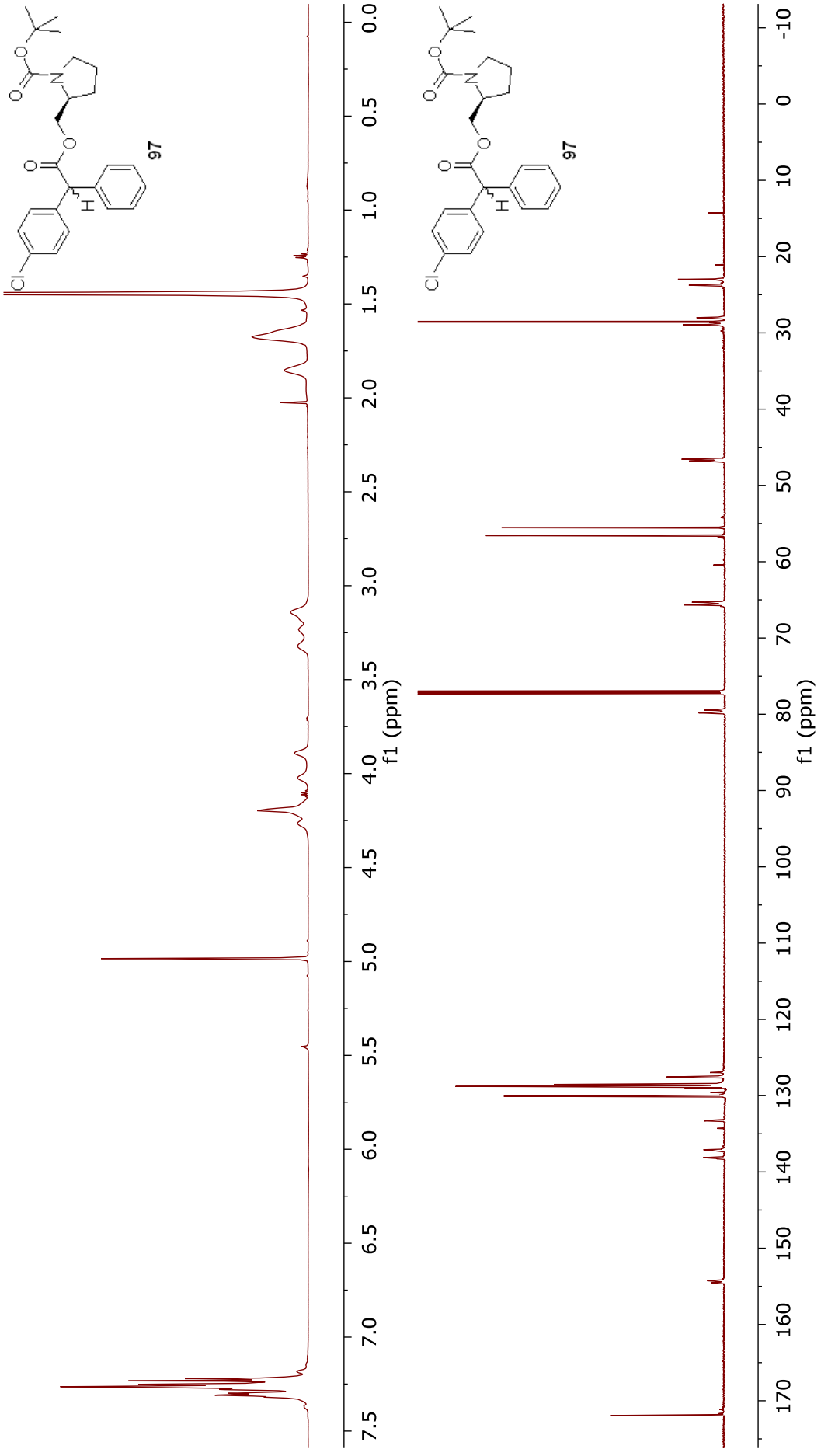
Note: Residual ethyl acetate peaks at δ_H = 4.12, 2.04 and 1.26 ppm; δ_C = 171.3, 60.5, 21.2 and 14.3 ppm

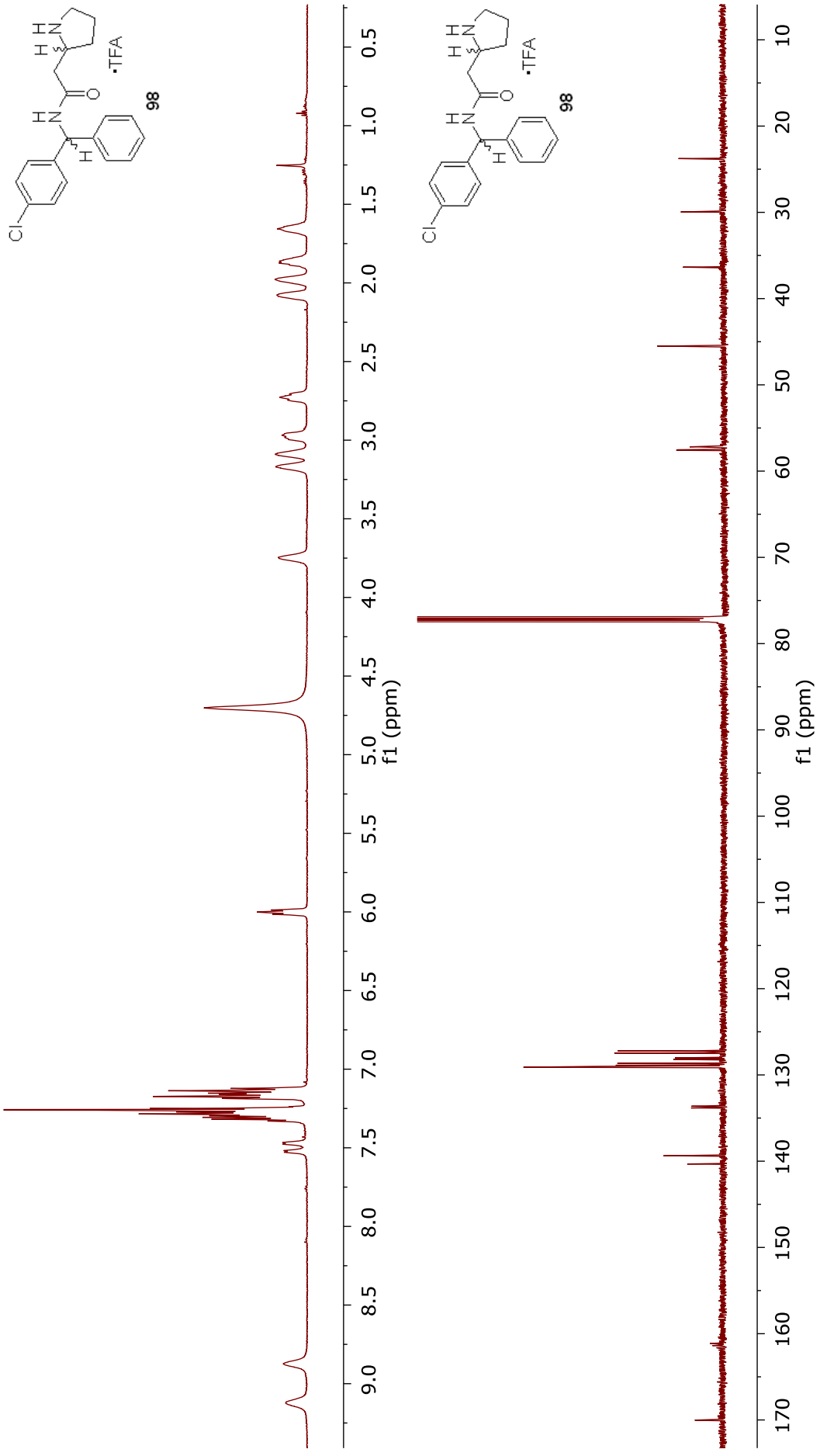


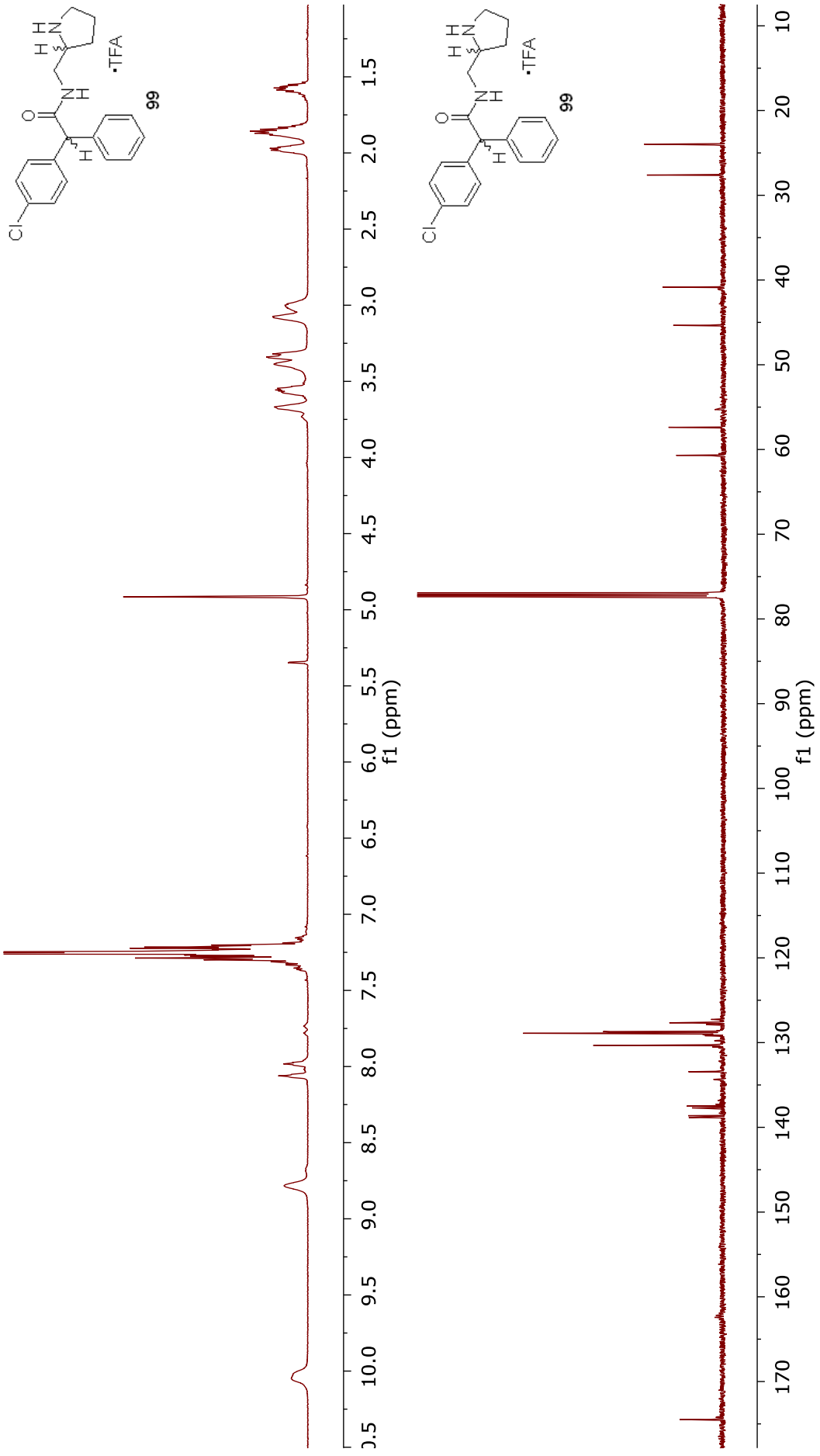
Note: Residual ethyl acetate peaks at δ_H = 4.12, 2.04 and 1.26 ppm; δ_C = 171.3, 60.5, 21.2 and 14.3 ppm

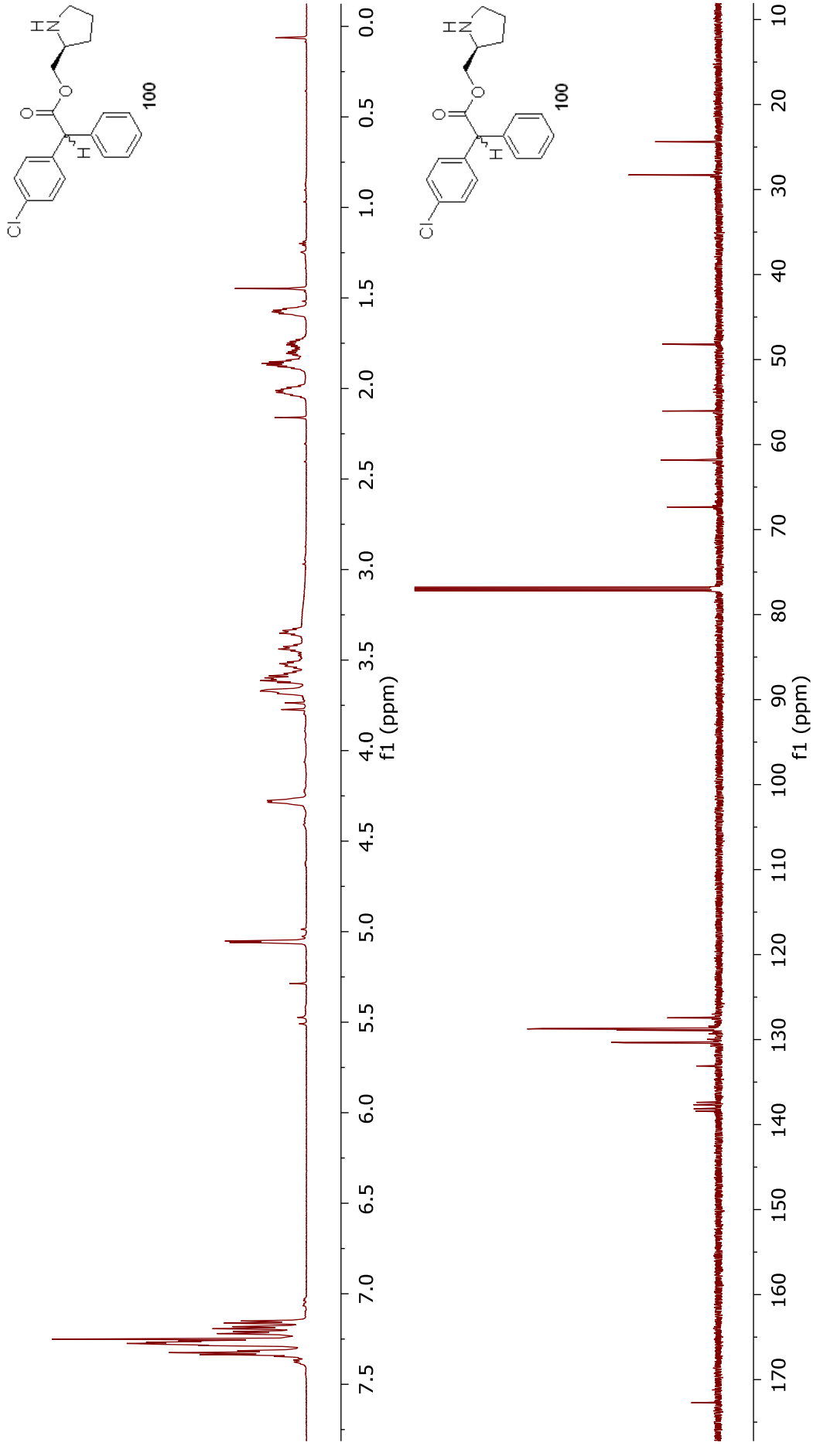


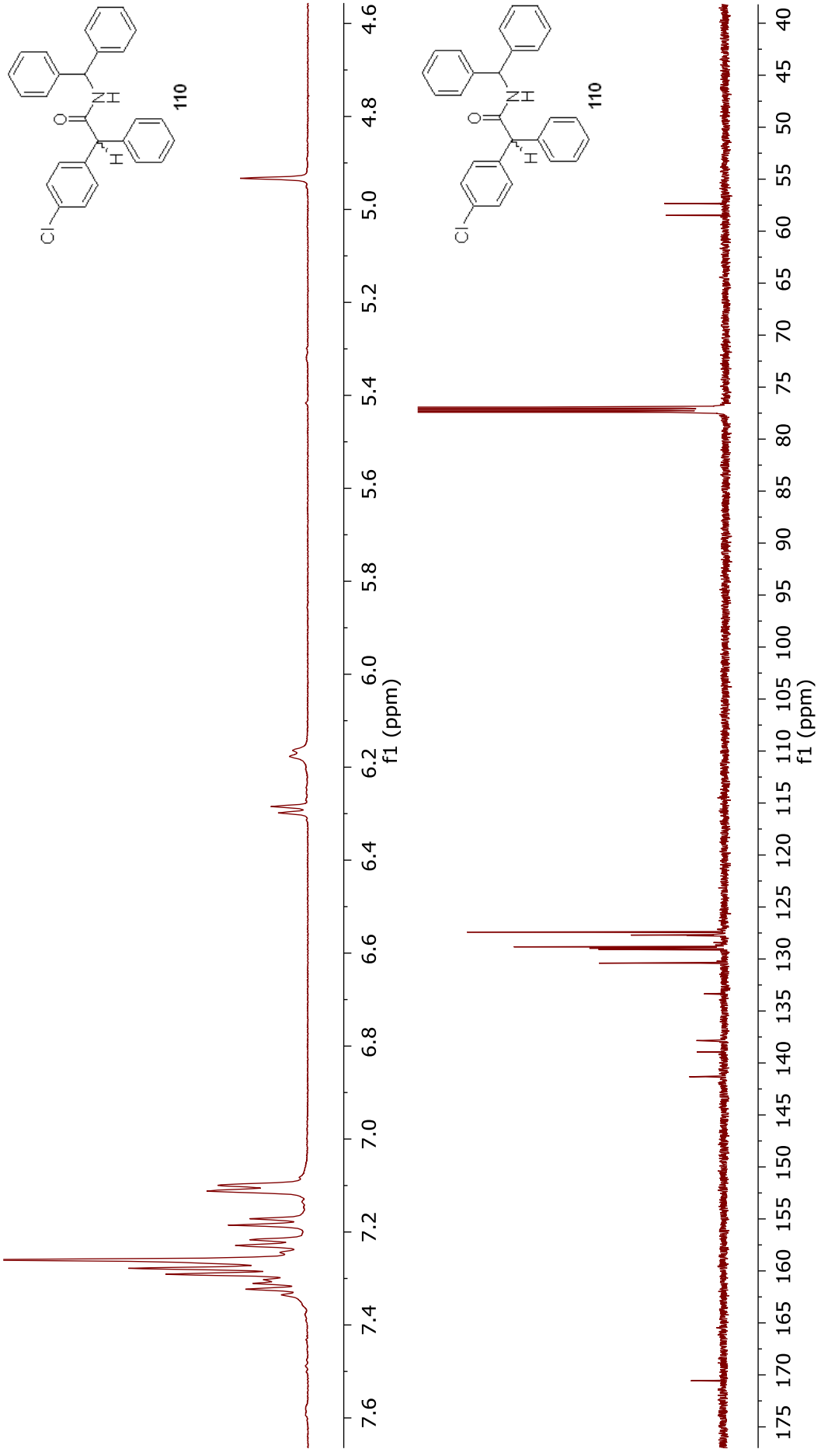
Note: Residual ethyl acetate peaks at δ_H = 4.12, 2.04 and 1.26 ppm; δ_C = 171.3, 60.5, 21.2 and 14.3 ppm

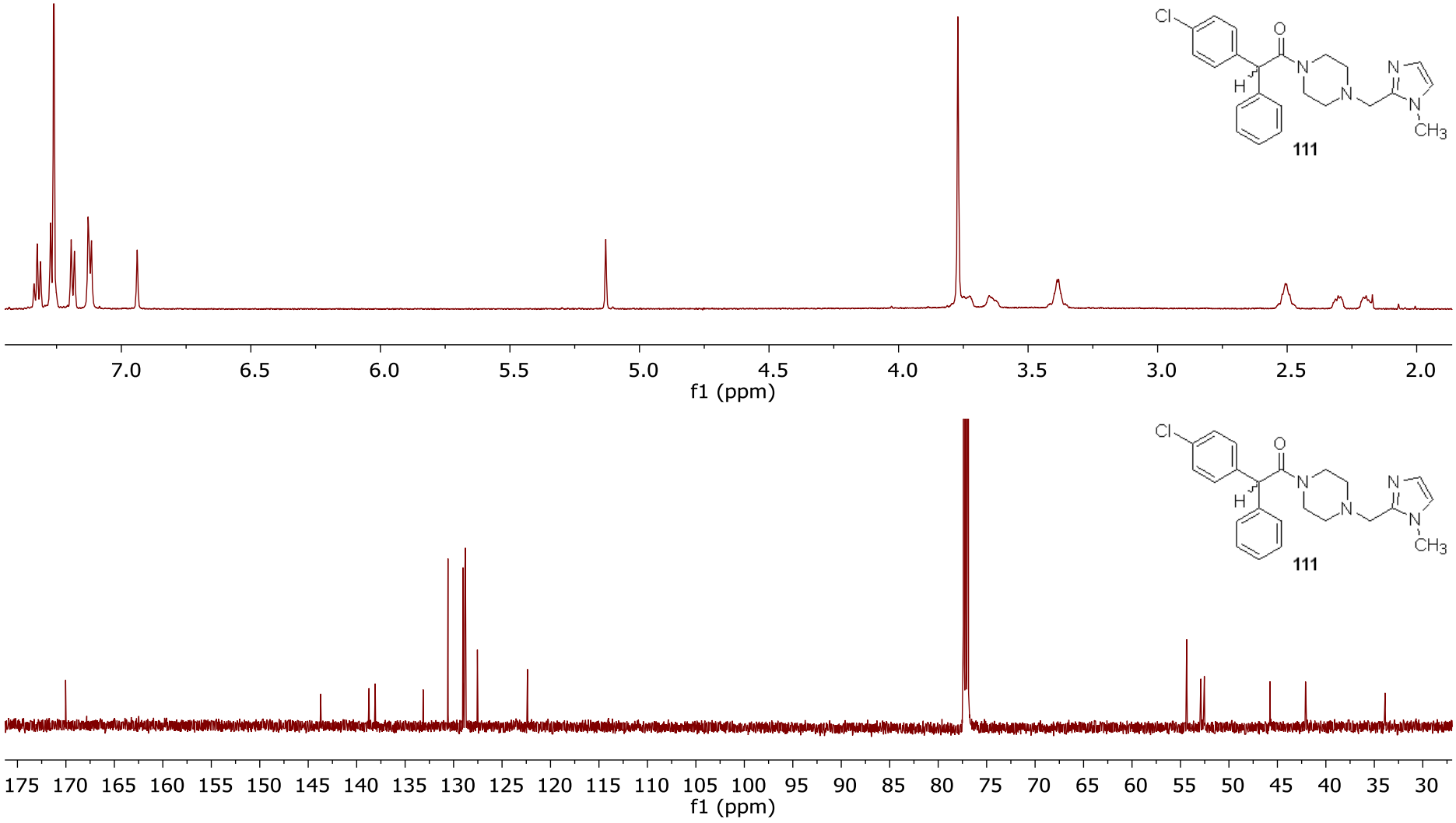

97
f1 (ppm)
97
f1 (ppm)

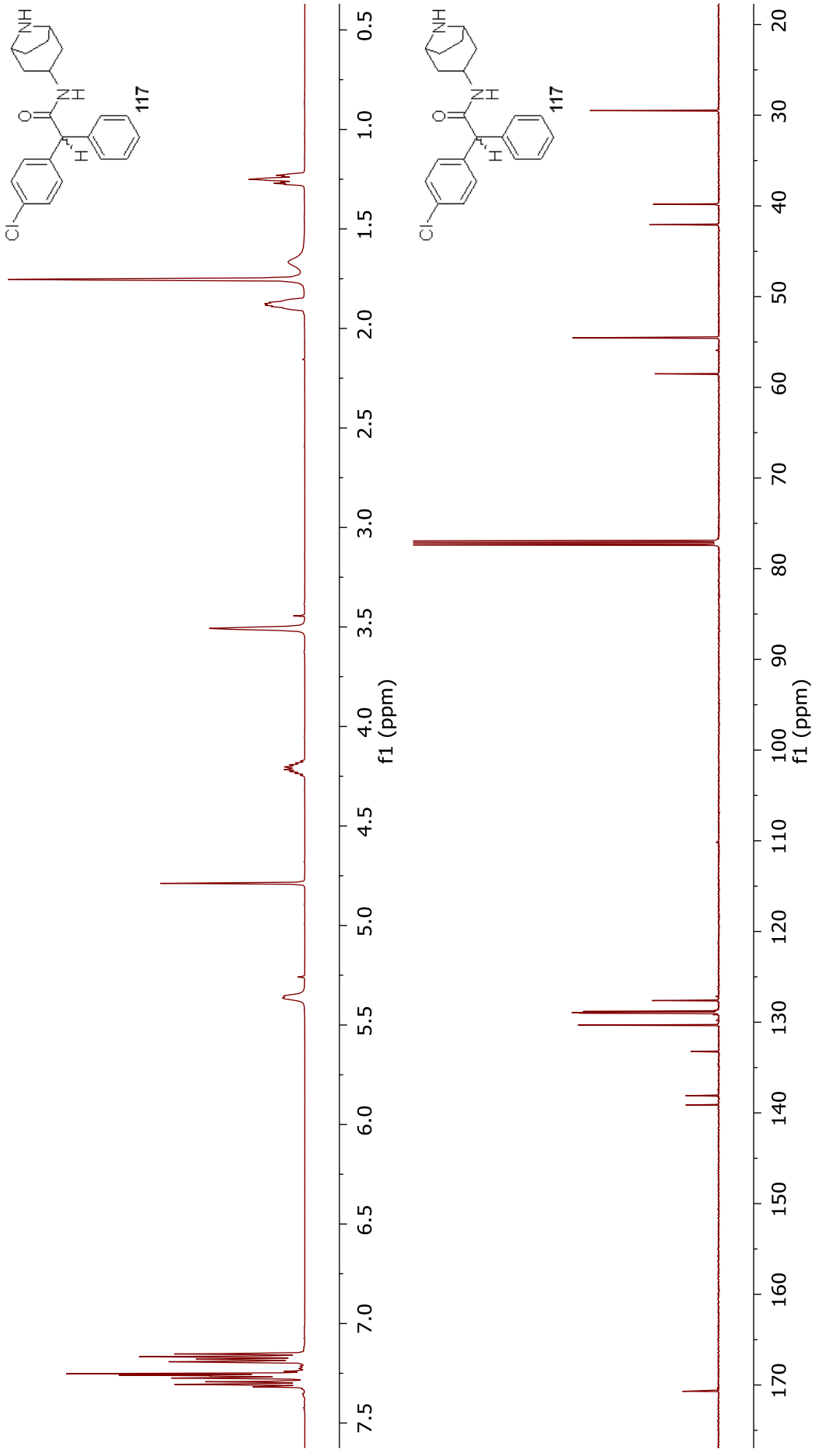

·TFA
98
f1 (ppm)
·TFA
98
f1 (ppm)

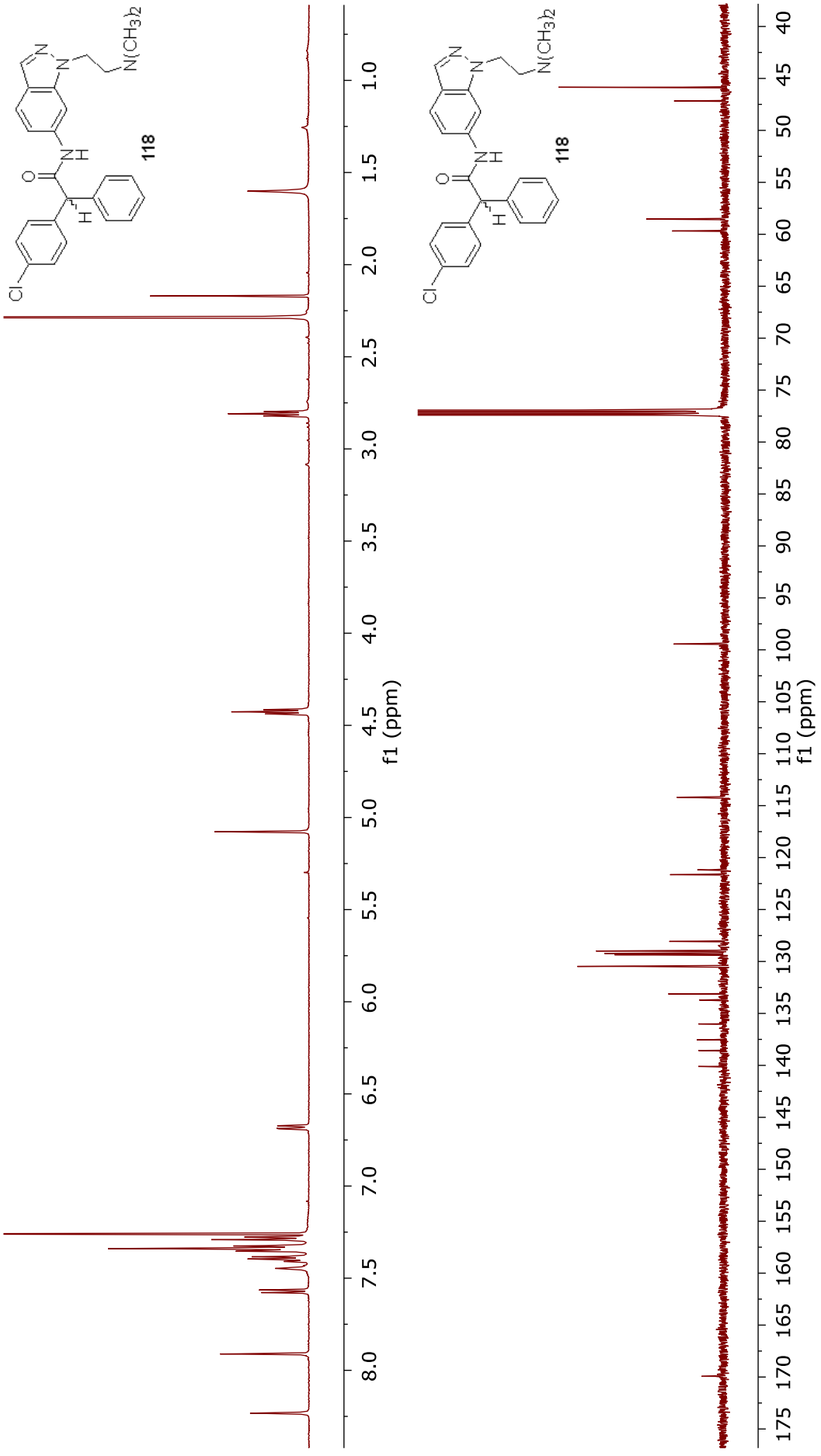

99
·TFA
f1 (ppm)
99
·TFA
f1 (ppm)

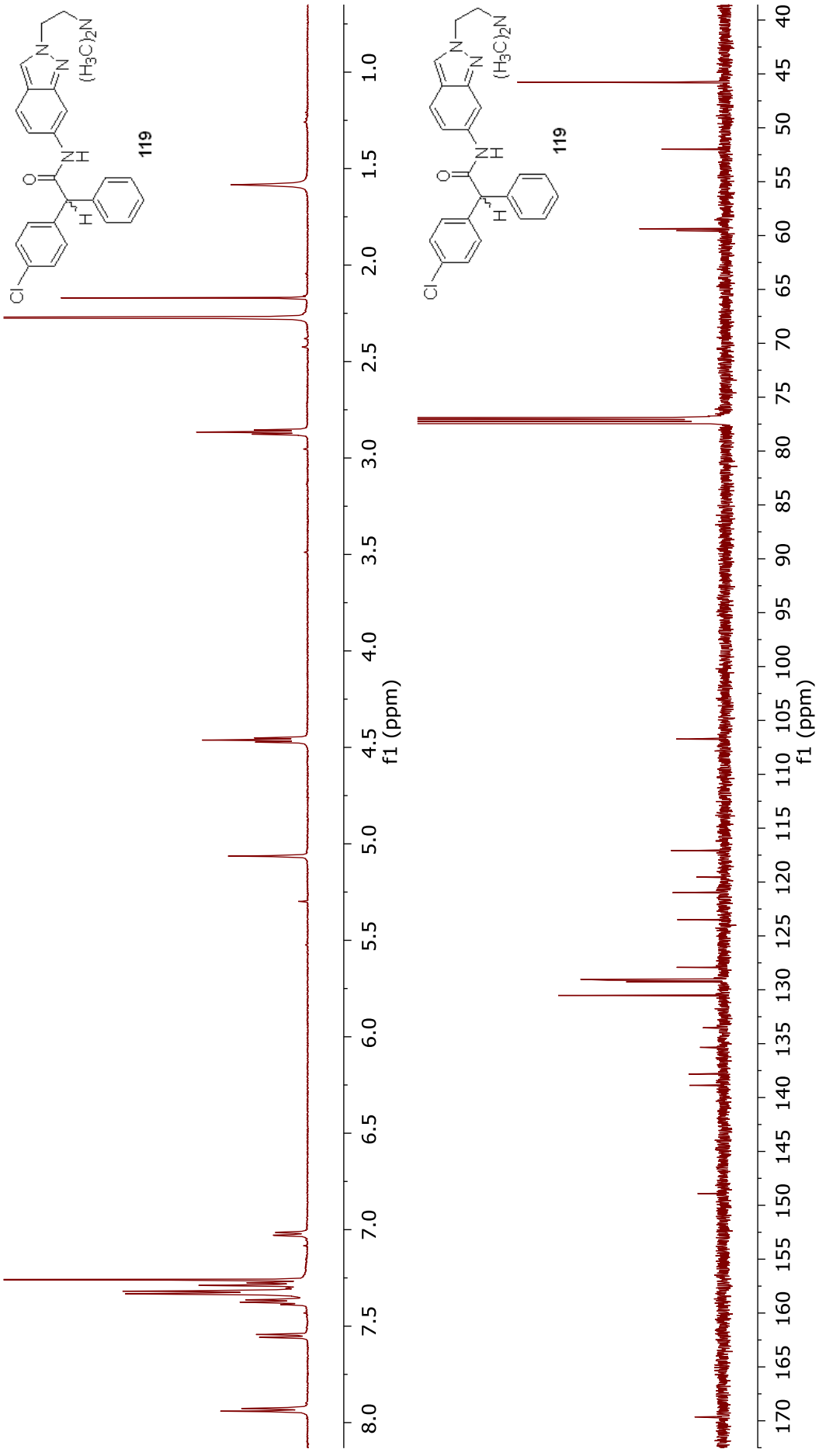

100
f1 (ppm)
100
f1 (ppm)

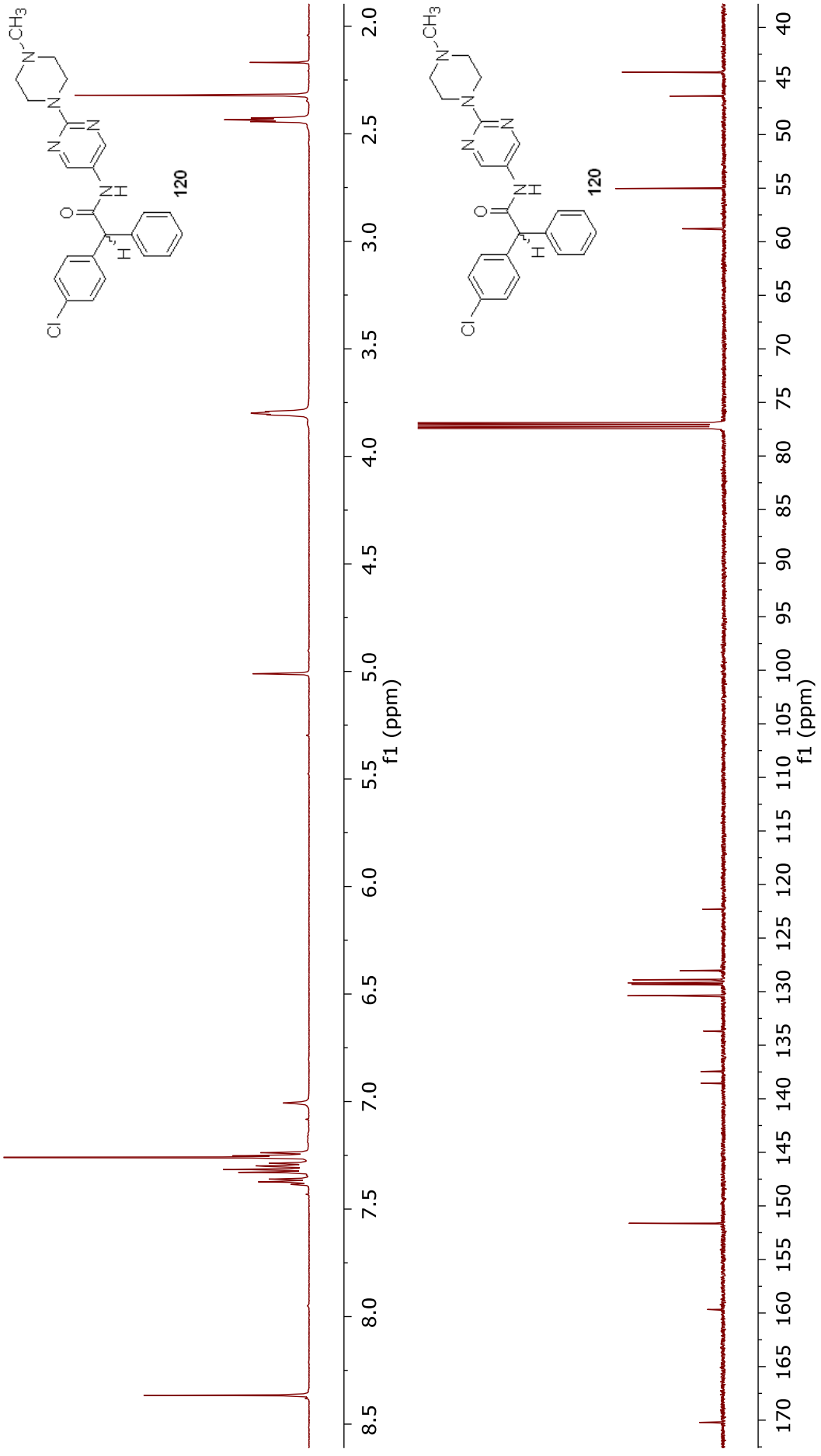

110
f1 (ppm)
110
f1 (ppm)

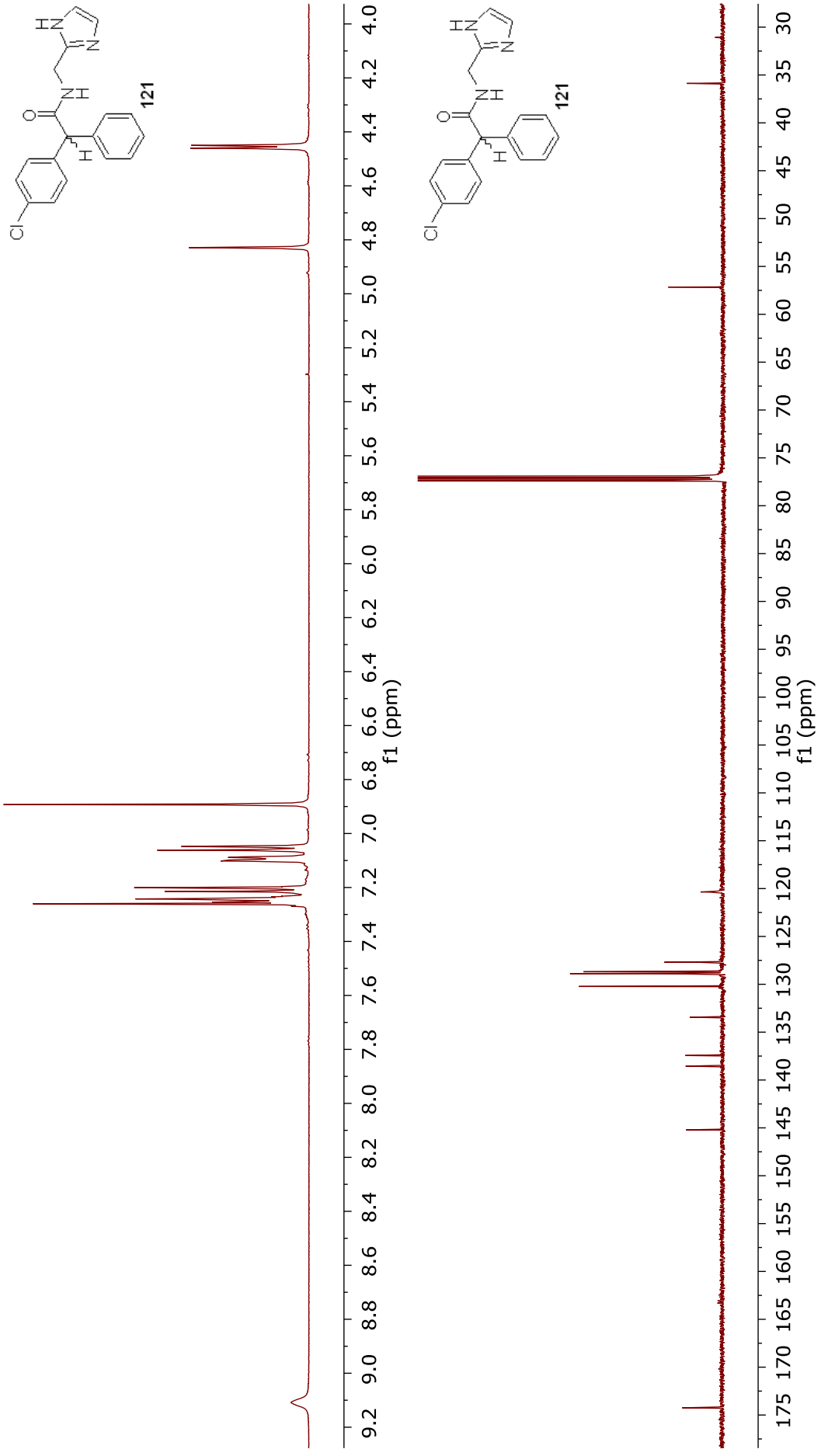

Cl
O
N
N
N
N
CH3
H
111
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f1 (ppm)
Cl
O
N
N
N
N
CH3
H
111
175 170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30
f1 (ppm)

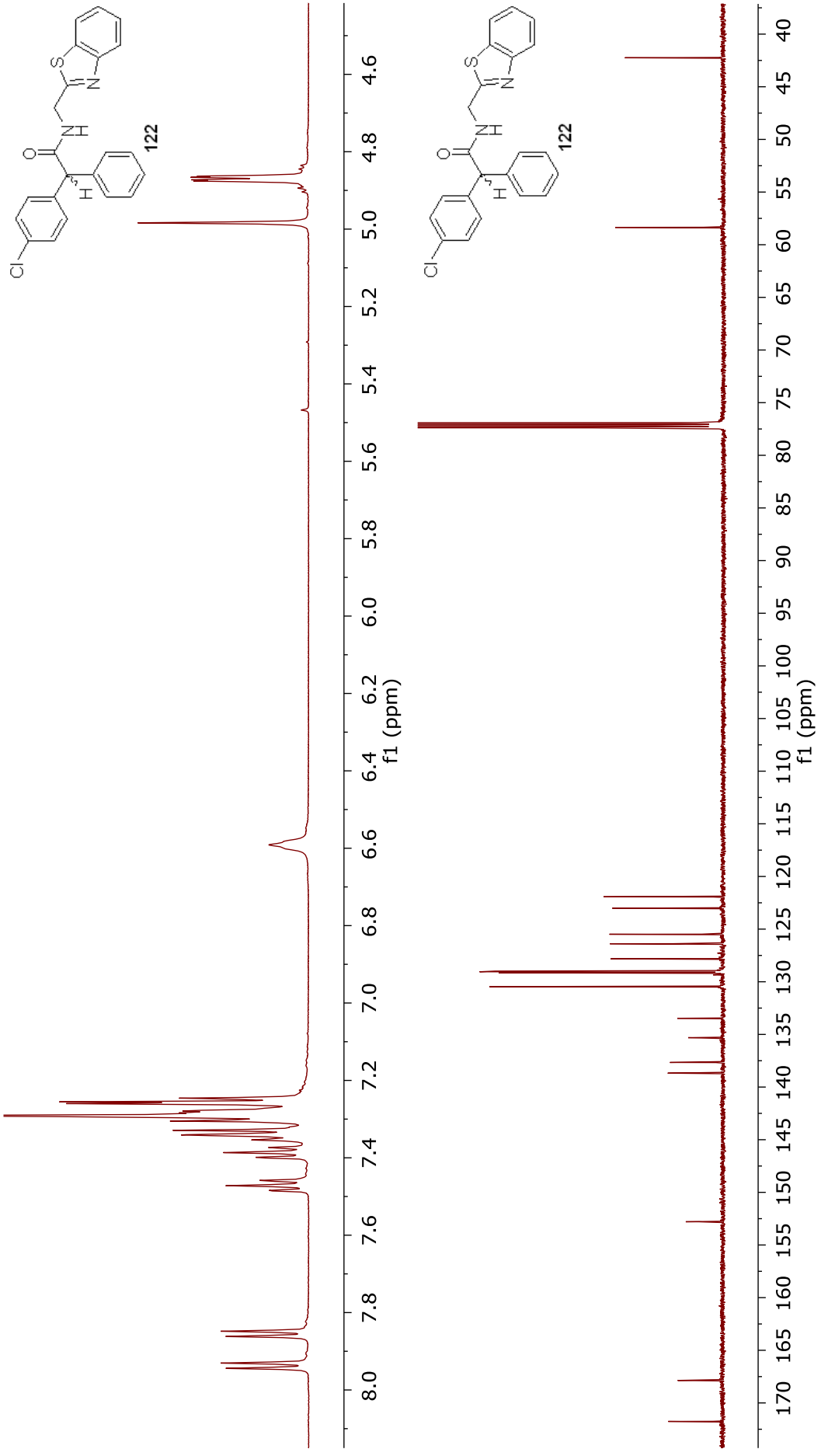

NH
NH
O
H
Cl
117
f1 (ppm)
NH
NH
O
H
Cl
117
f1 (ppm)

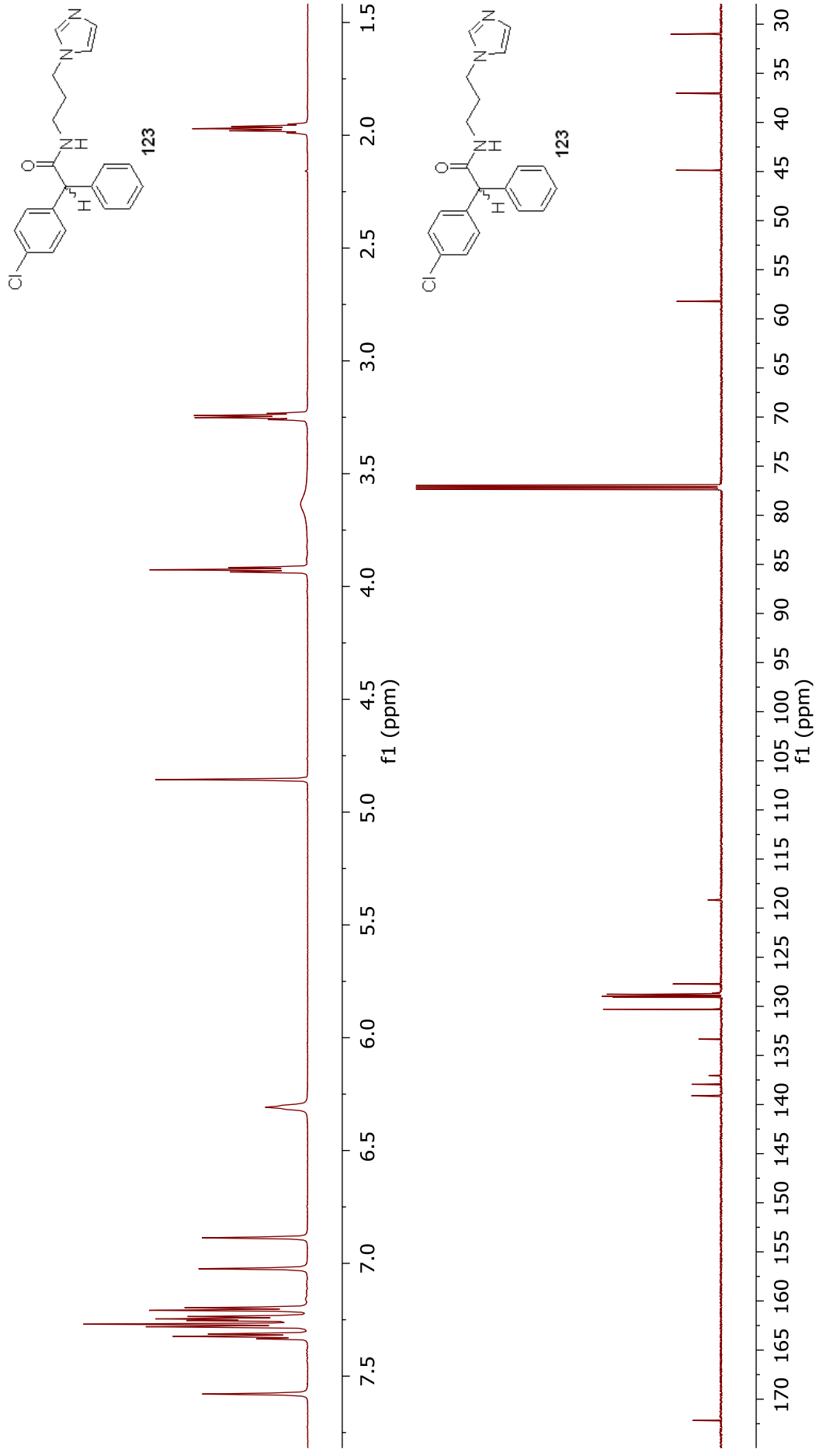

N(CH3)2
118
f1 (ppm)
N(CH3)2
118
f1 (ppm)

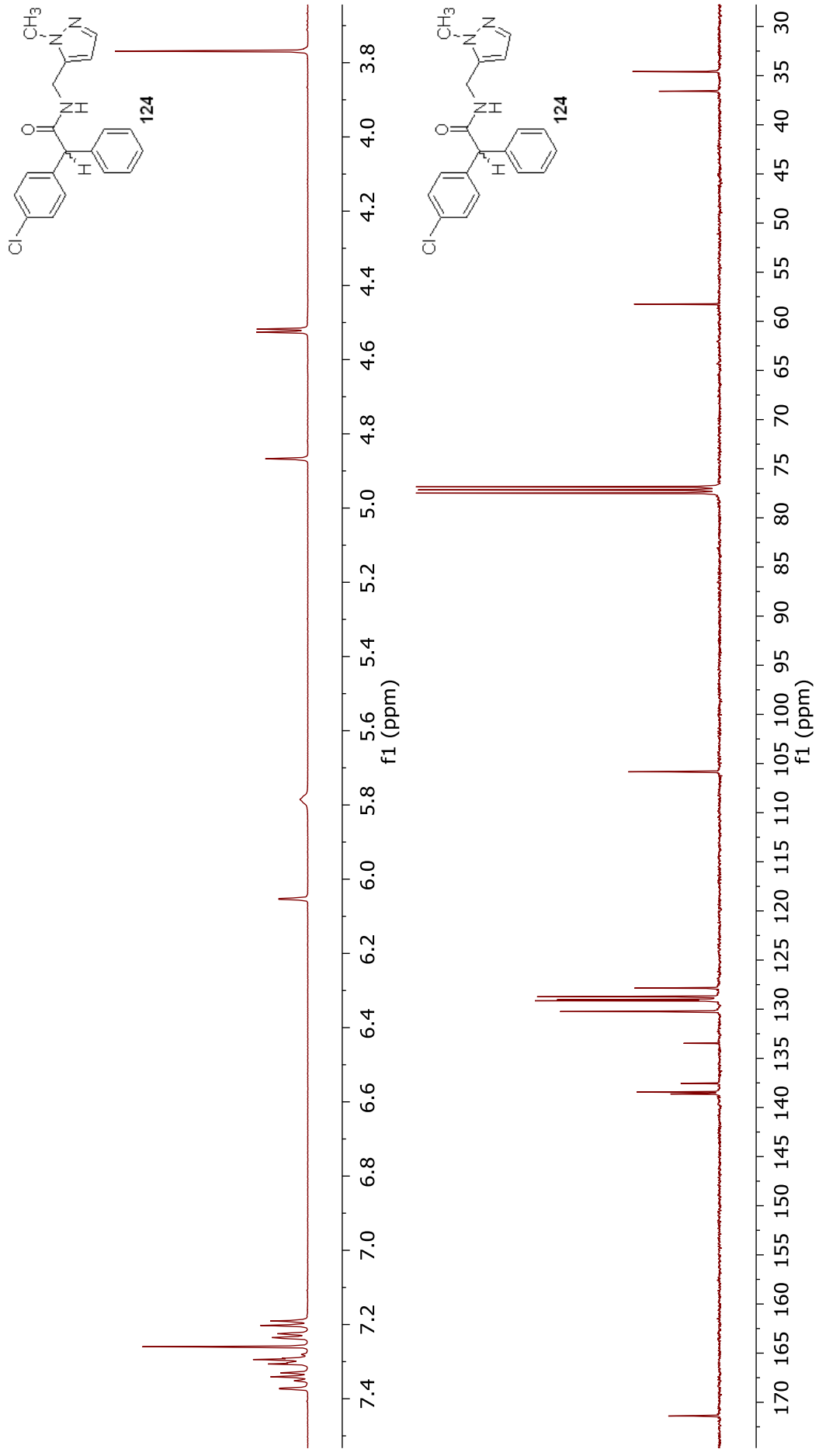

119
f1 (ppm)
119
f1 (ppm)

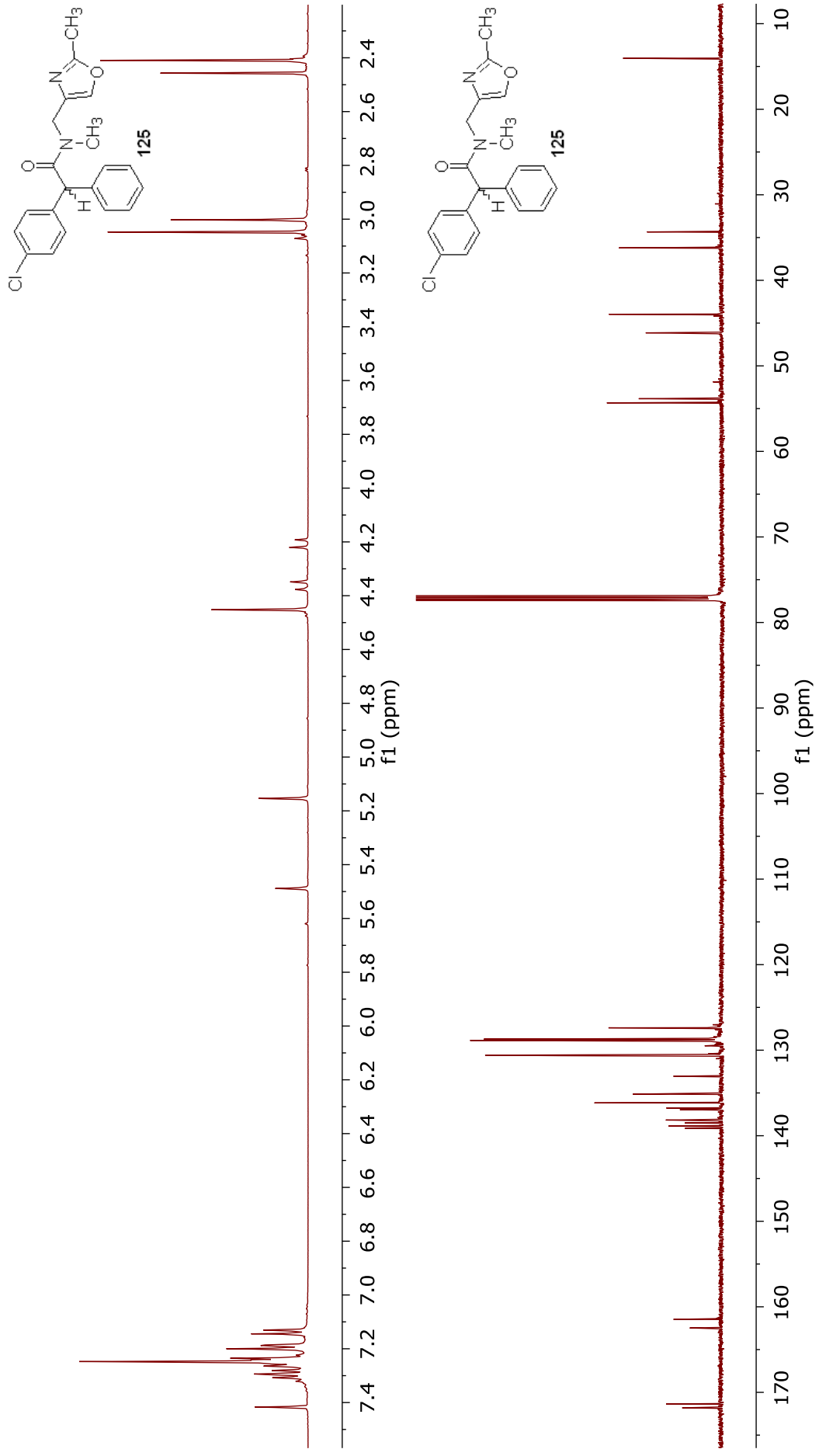

120
f1 (ppm)
120
f1 (ppm)

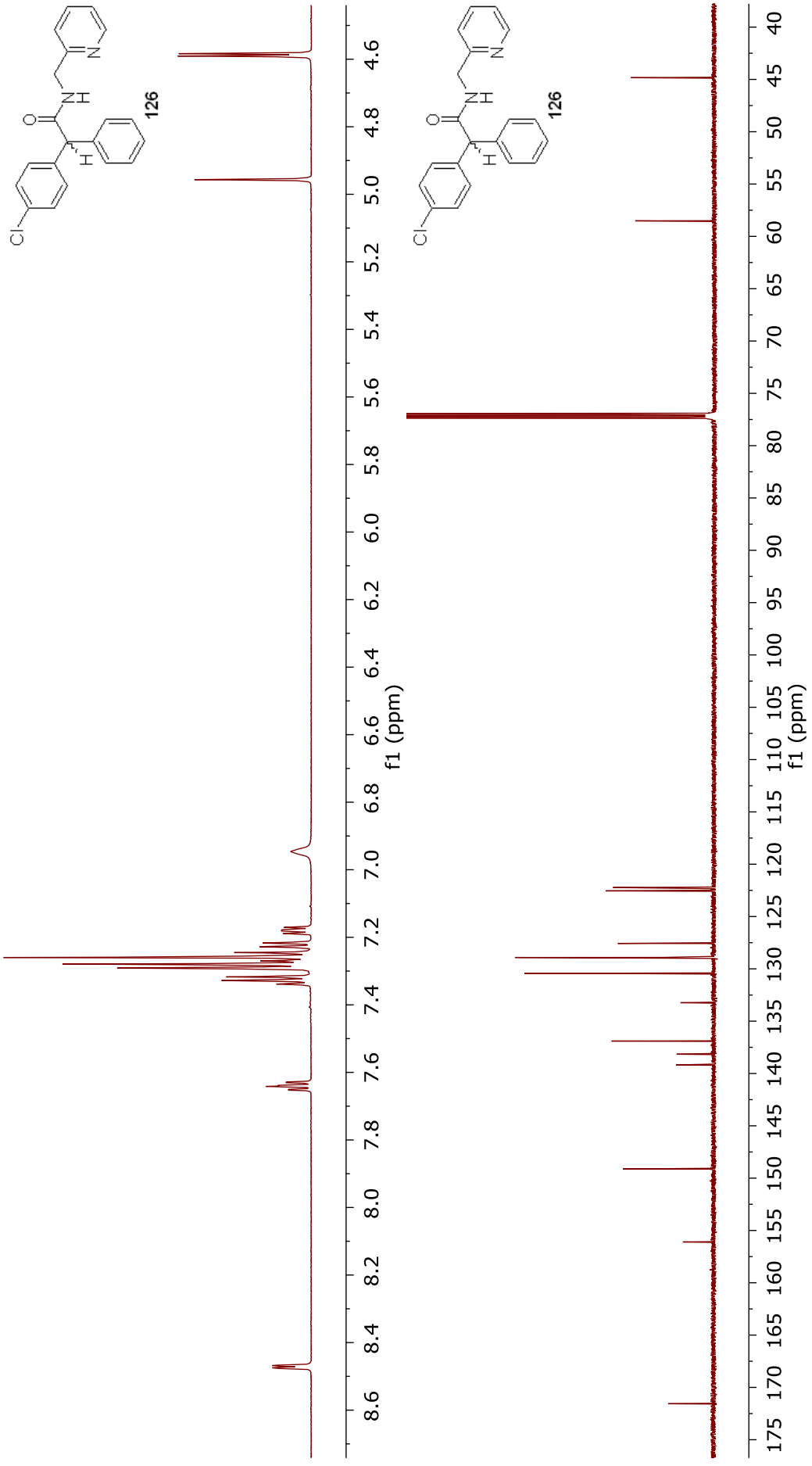

121
f1 (ppm)
121
f1 (ppm)

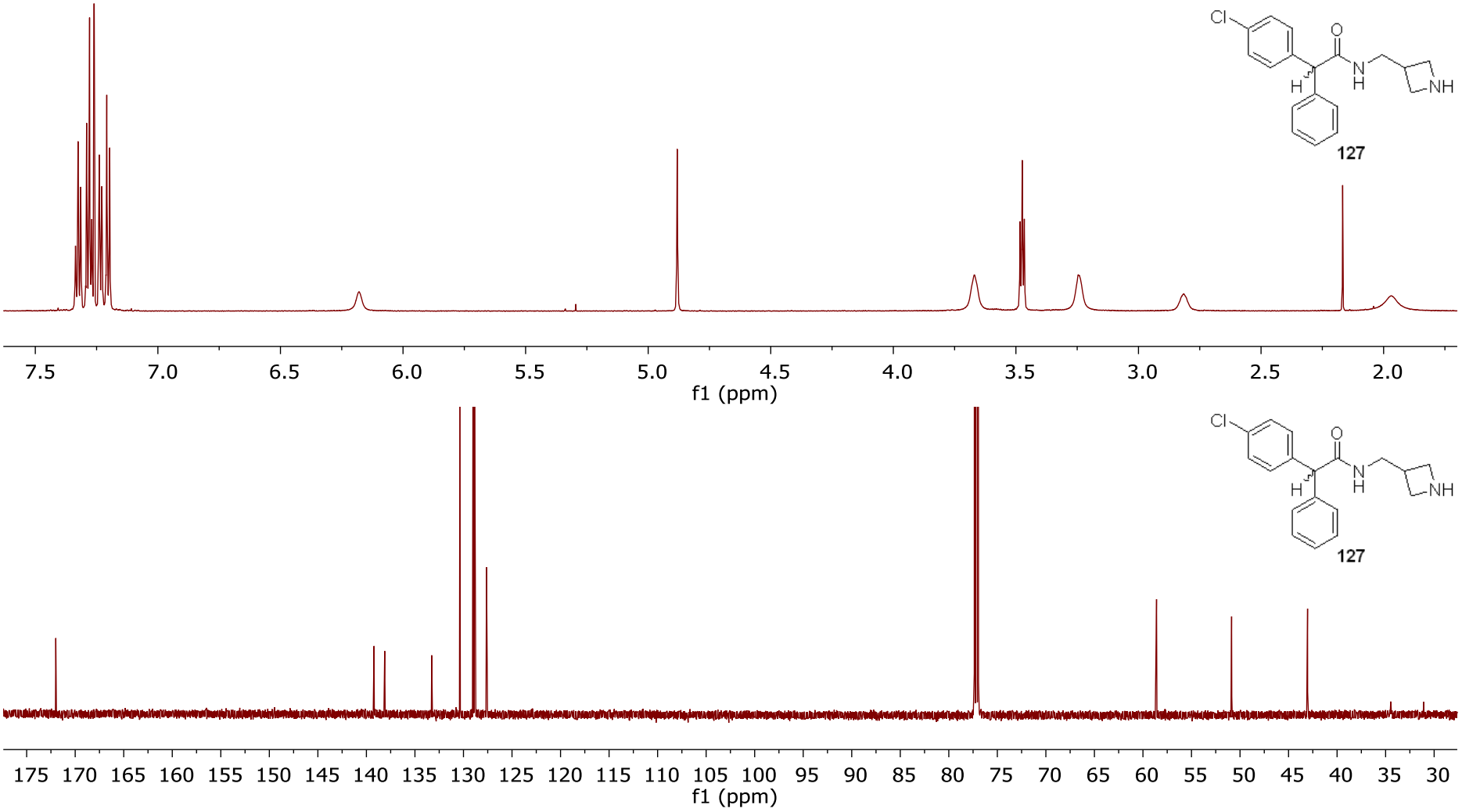

122
f1 (ppm)
122
f1 (ppm)


123
f1 (ppm)
123
f1 (ppm)

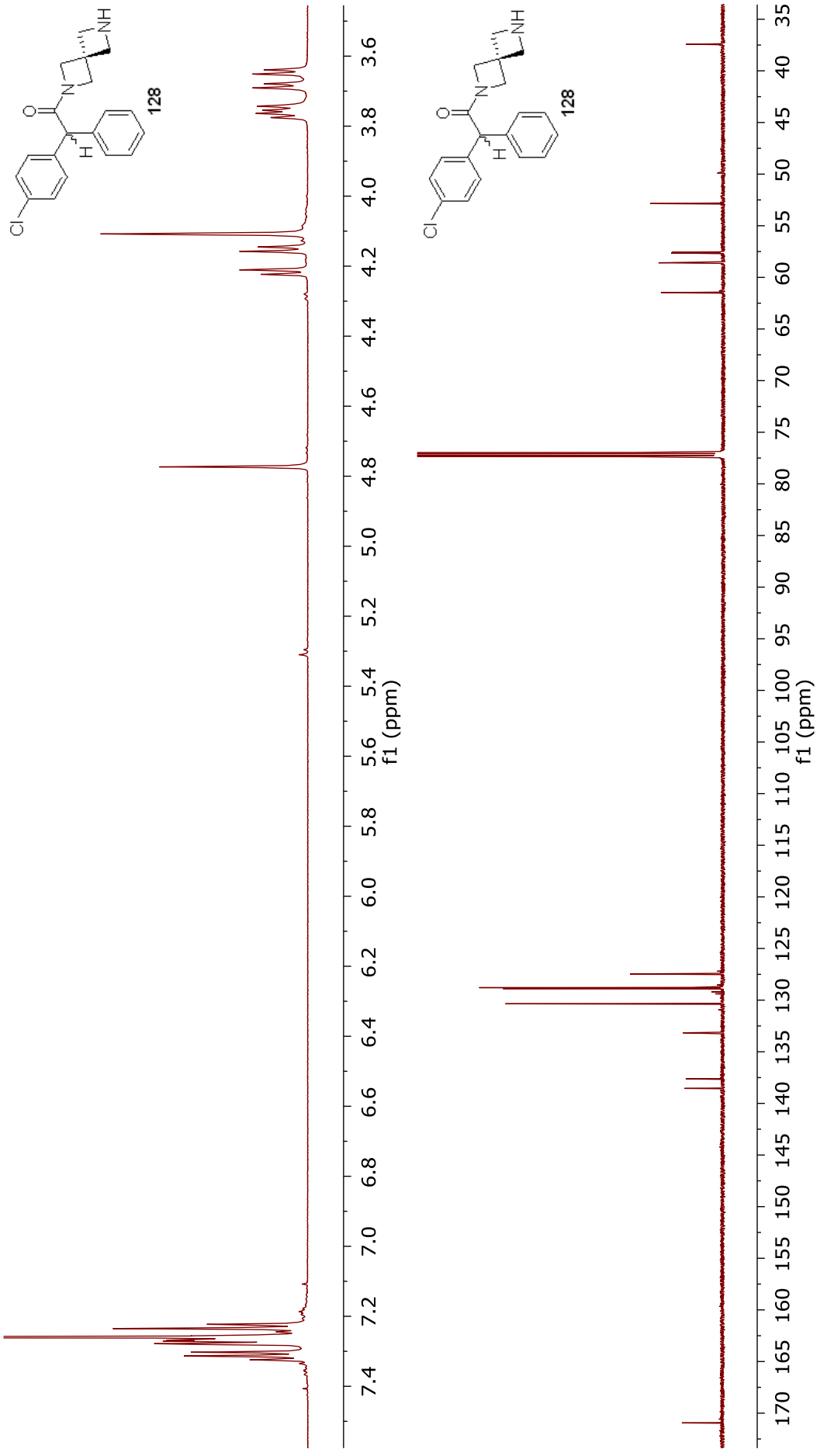

CH3
N
N
H
N
O
H
Cl
124
f1 (ppm)
CH3
N
N
H
N
O
H
Cl
124
f1 (ppm)

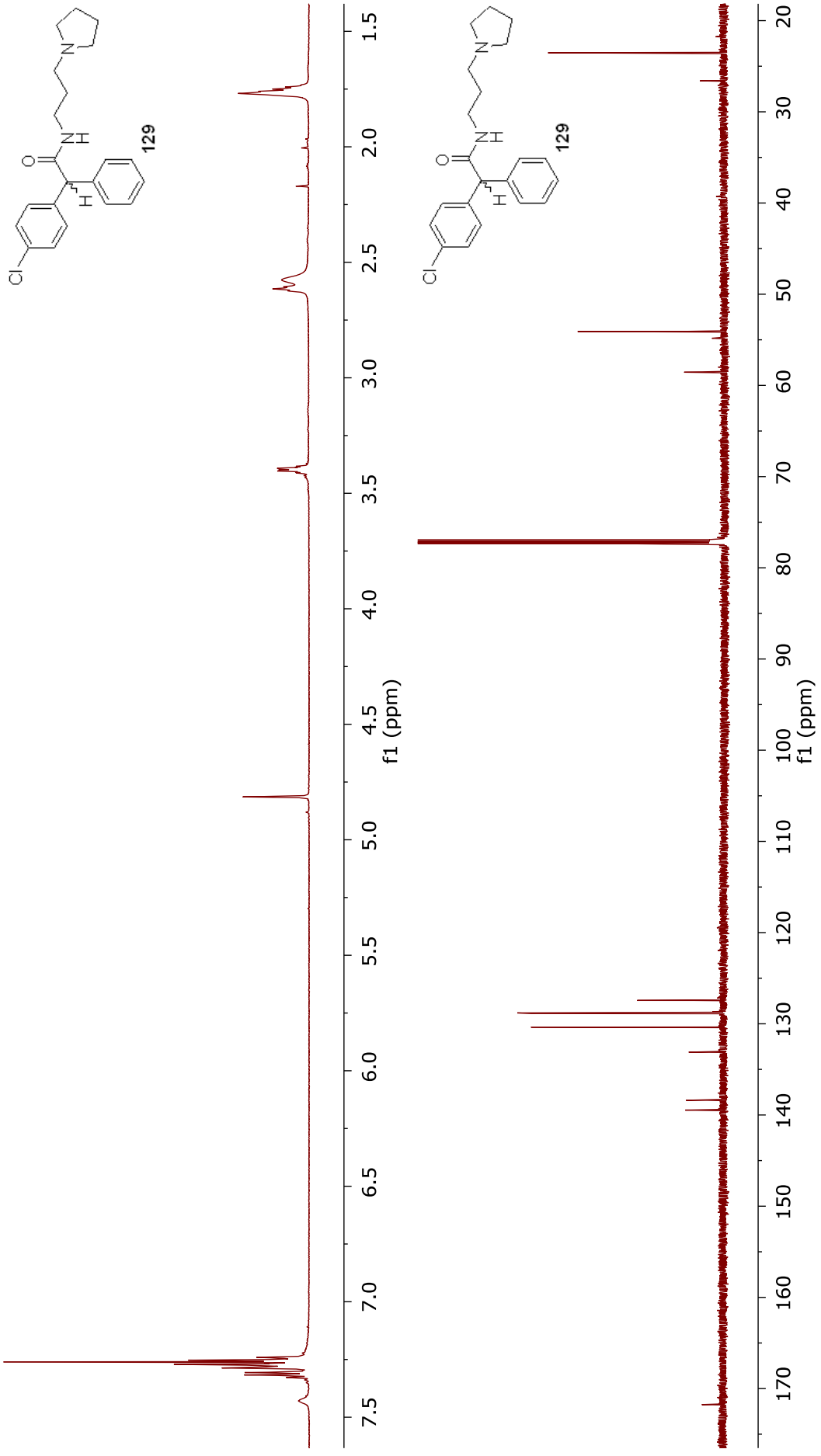

125
f1 (ppm)
125
f1 (ppm)

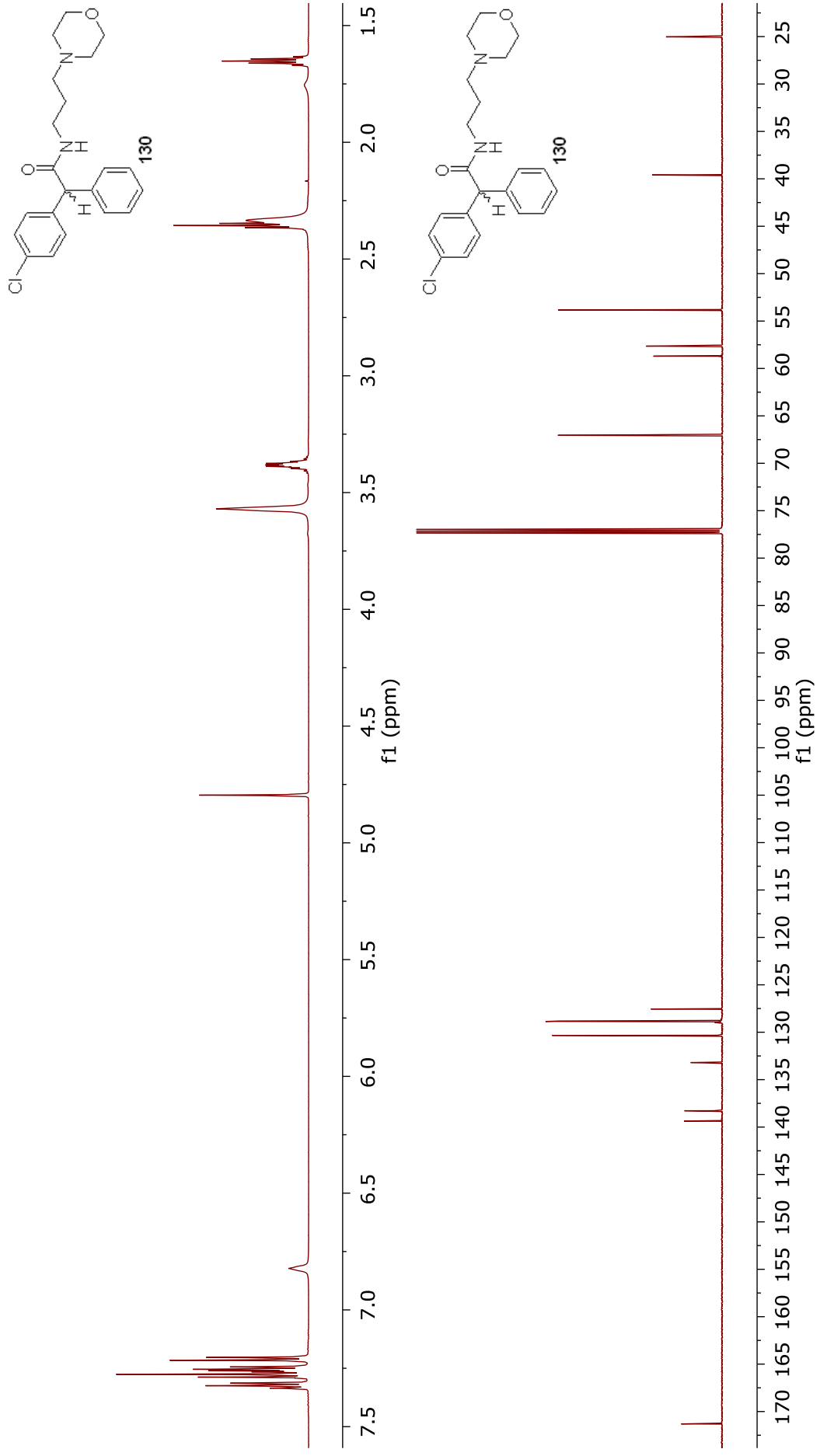

126
f1 (ppm)
126
f1 (ppm)

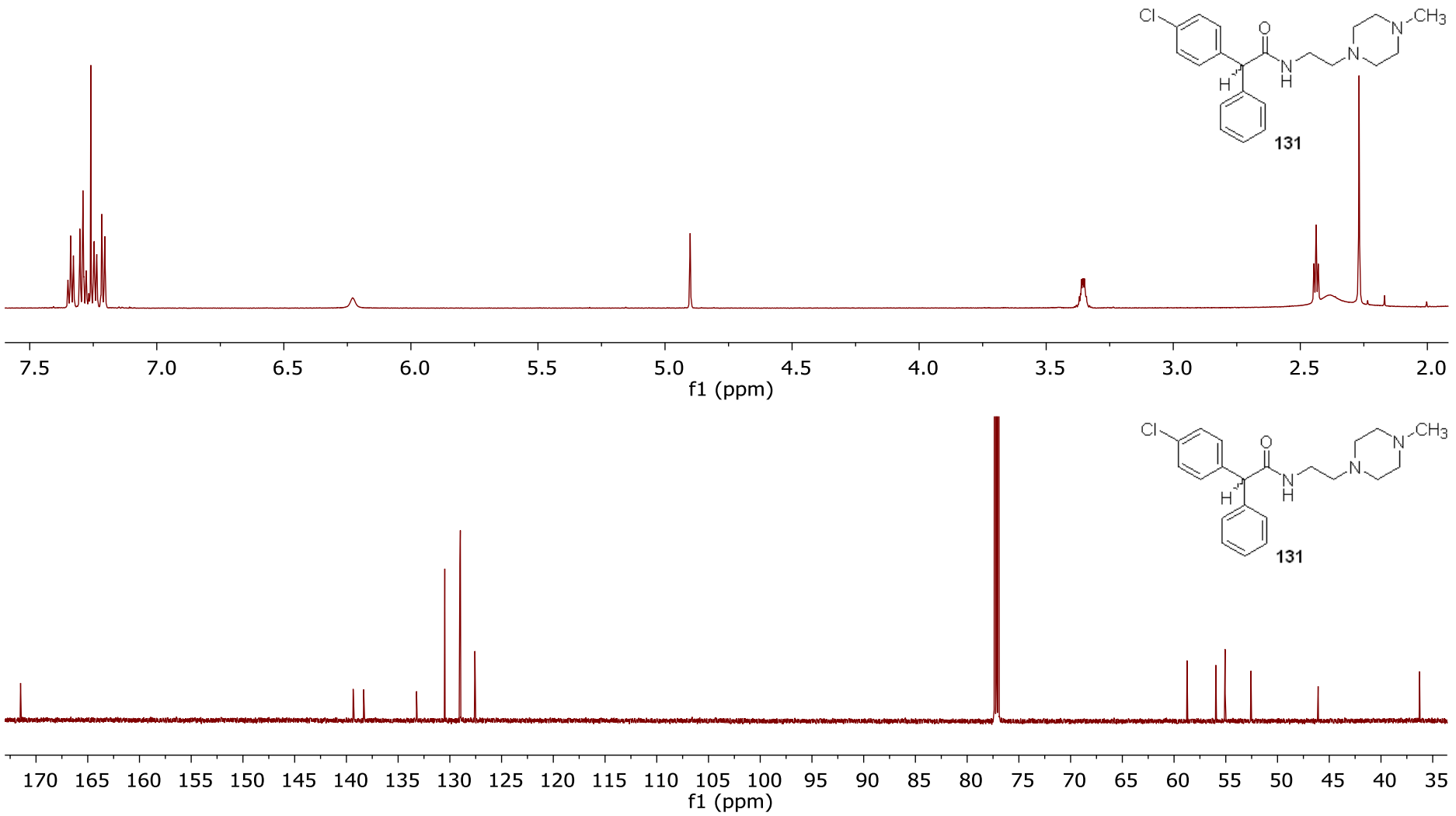


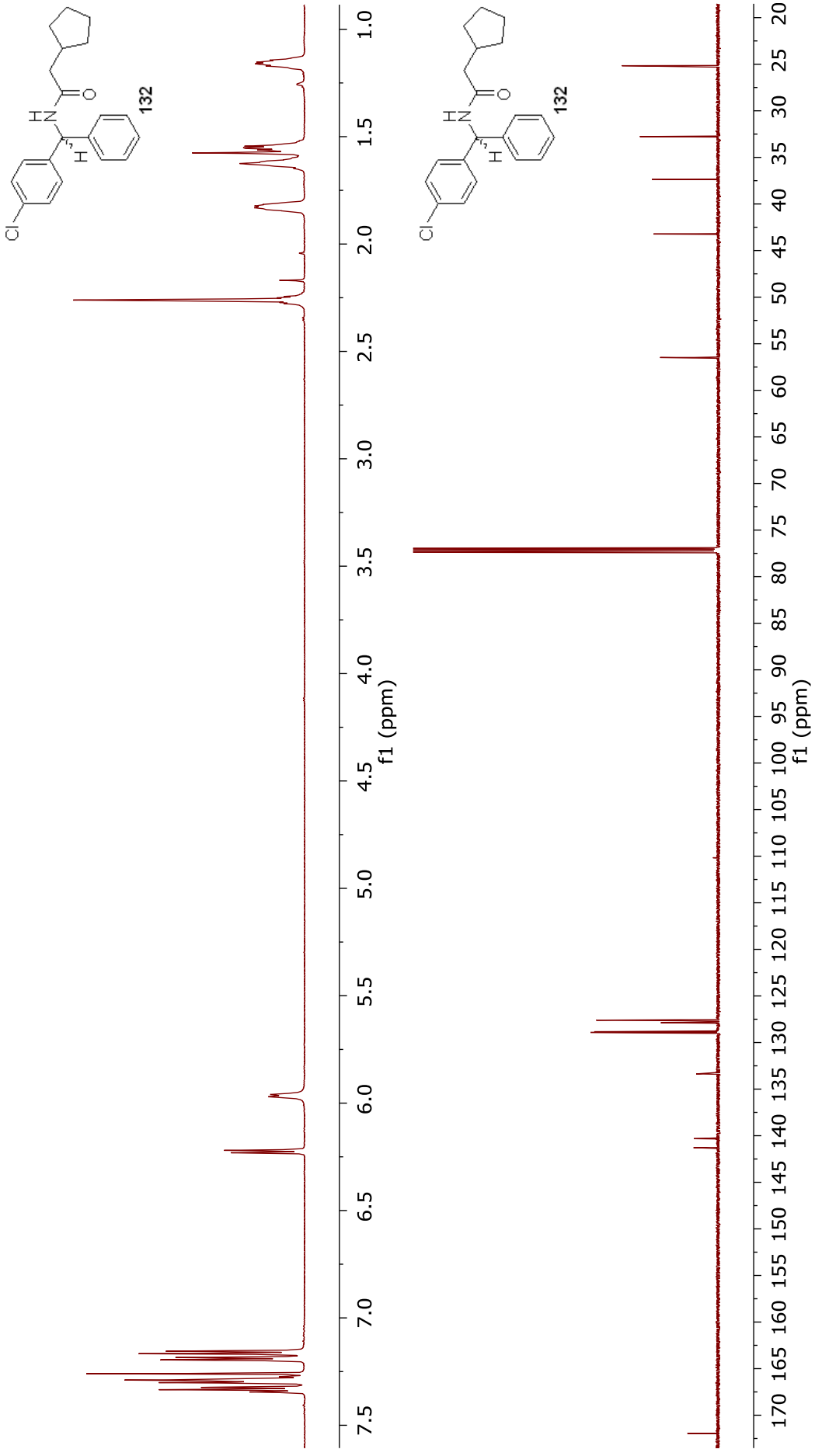
Note: Residual acetone peaks at δ_H = 2.17 ppm; δ_C = 207.0 and 31.0 ppm

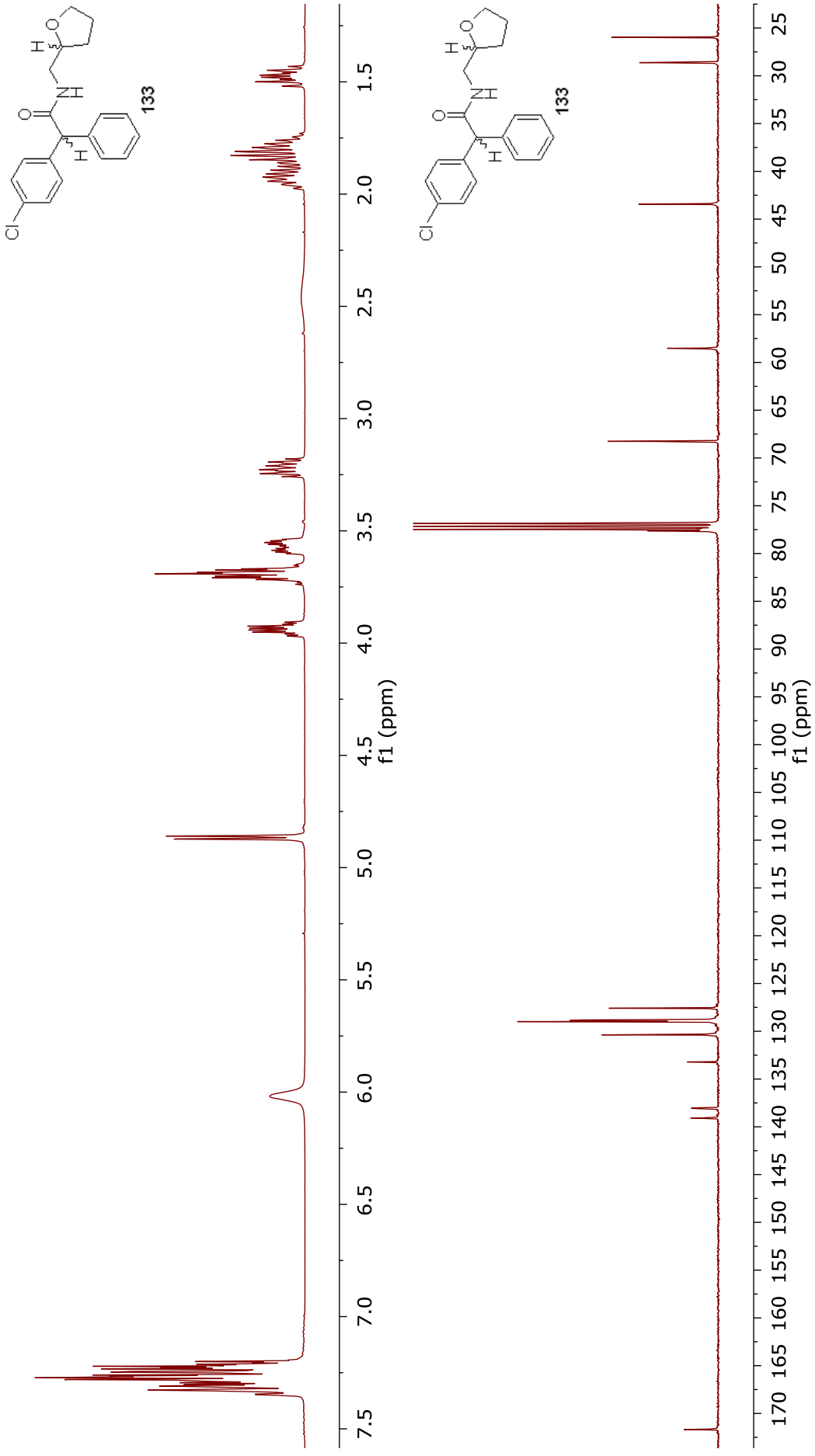

128
f1 (ppm)
128
f1 (ppm)

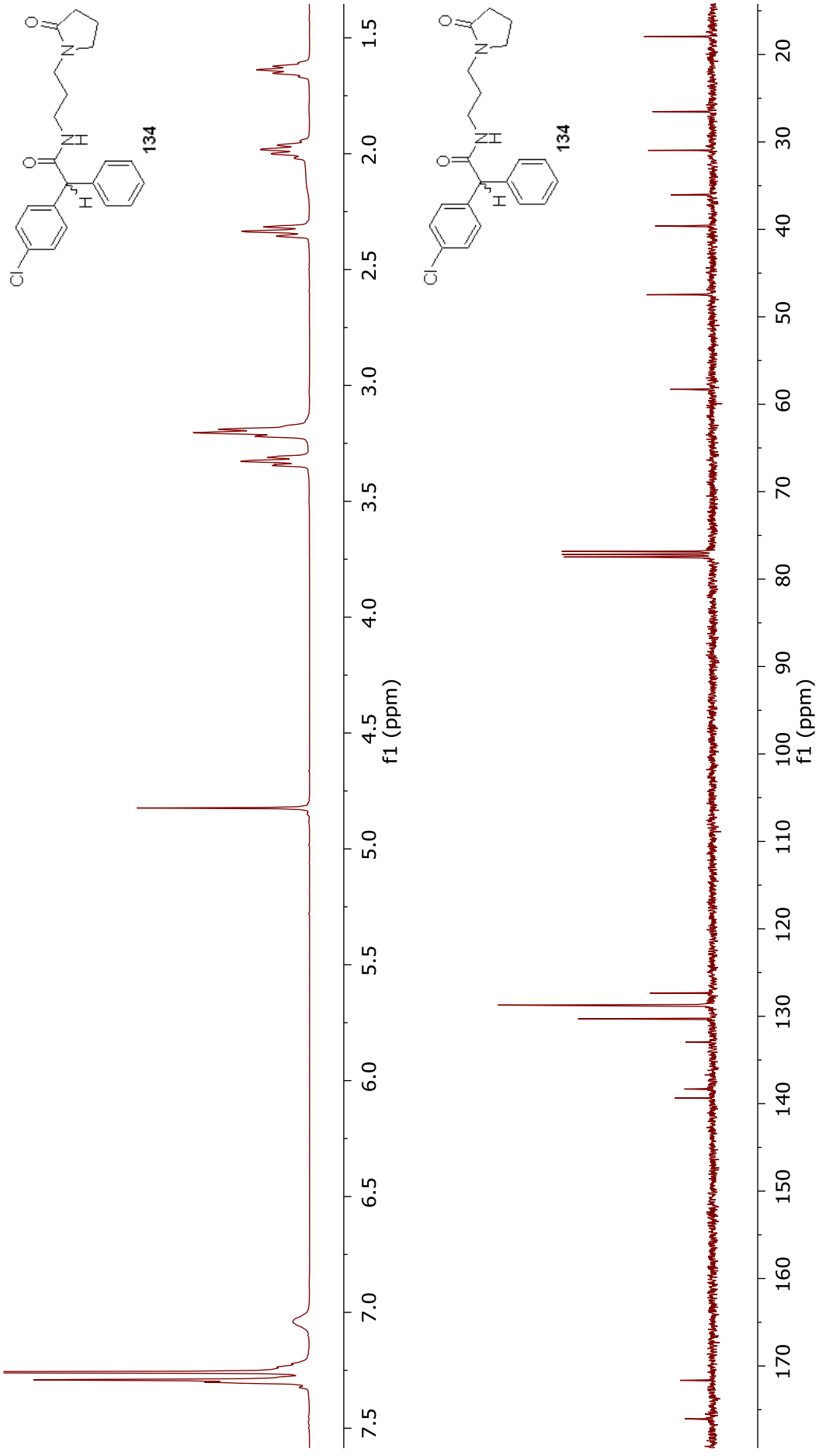

129
f1 (ppm)
129
f1 (ppm)

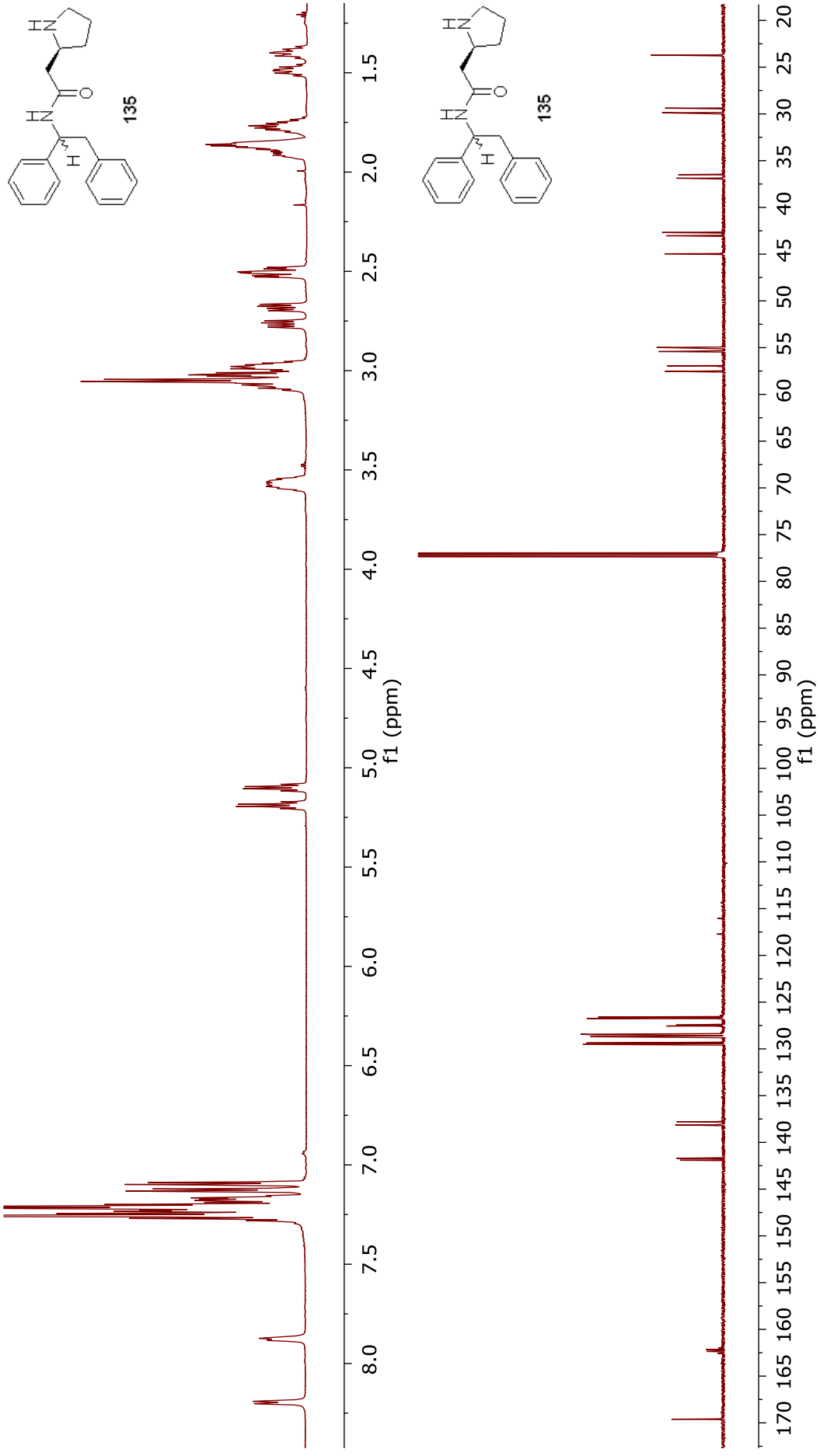

130
f1 (ppm)
130
f1 (ppm)

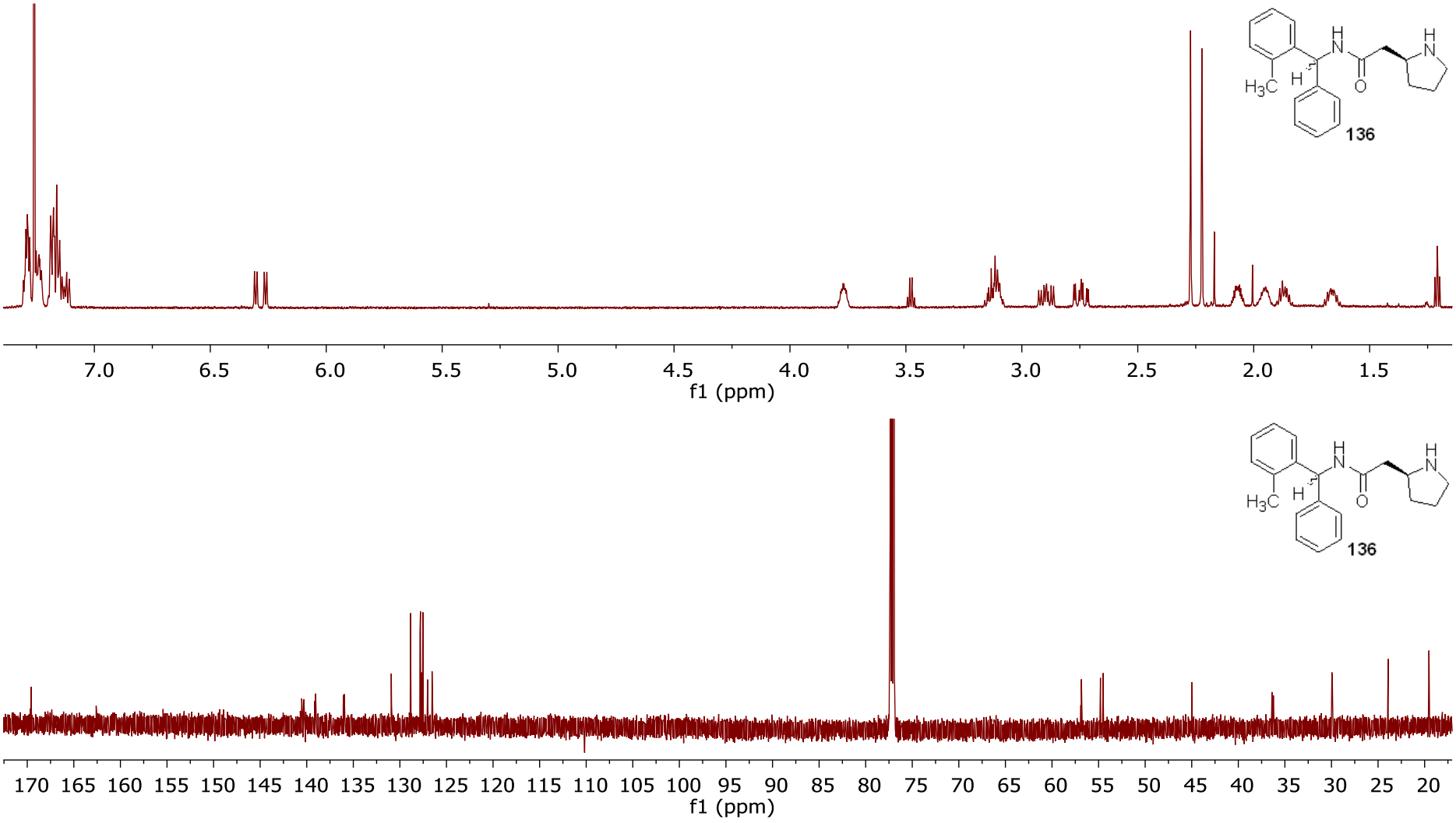

Cl
O
N
H
H
N
CH3
131
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0
f1 (ppm)
Cl
O
N
H
H
N
CH3
131
170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35
f1 (ppm)


132
f1 (ppm)
132
f1 (ppm)

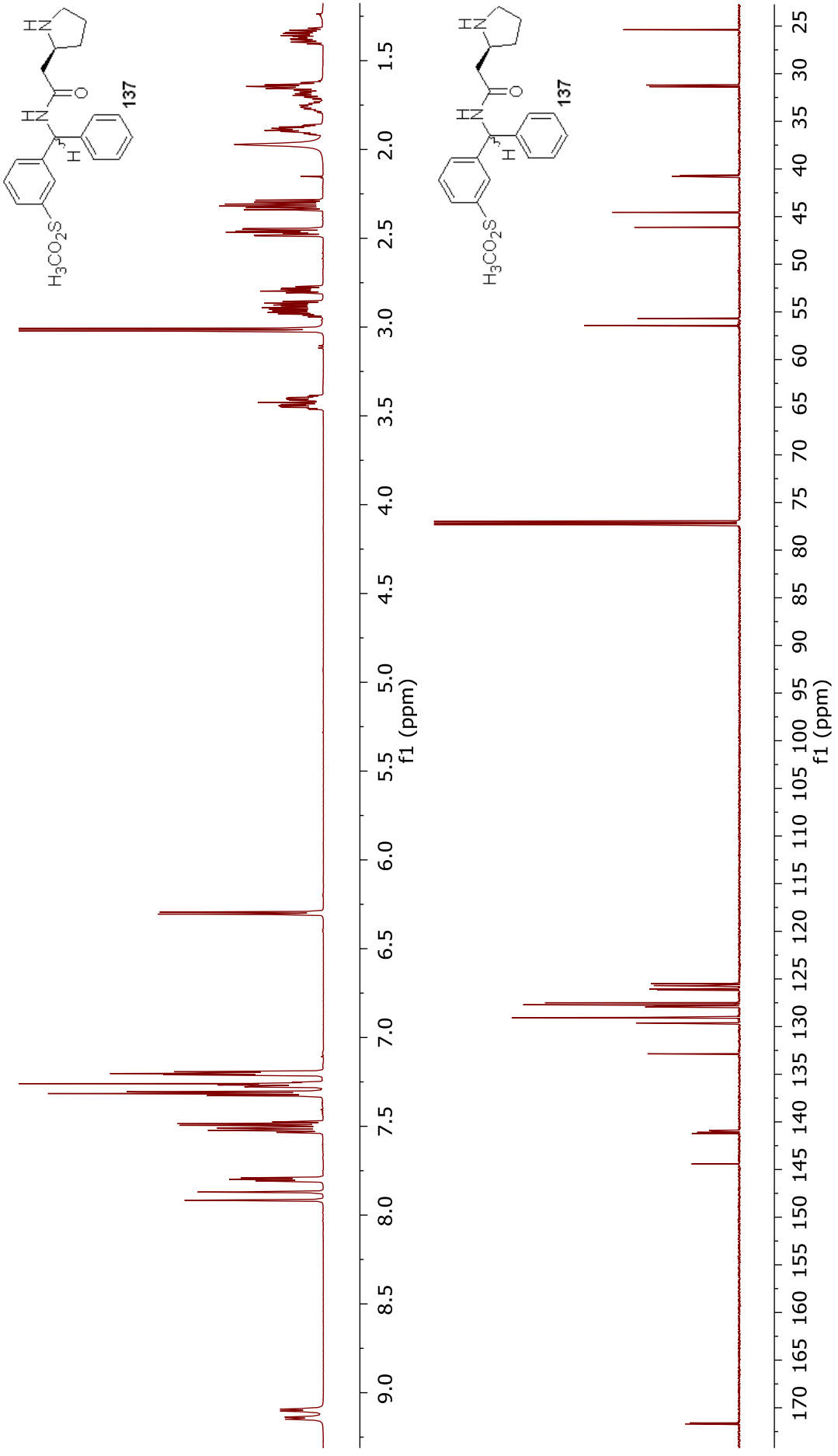

133
f1 (ppm)
133
f1 (ppm)

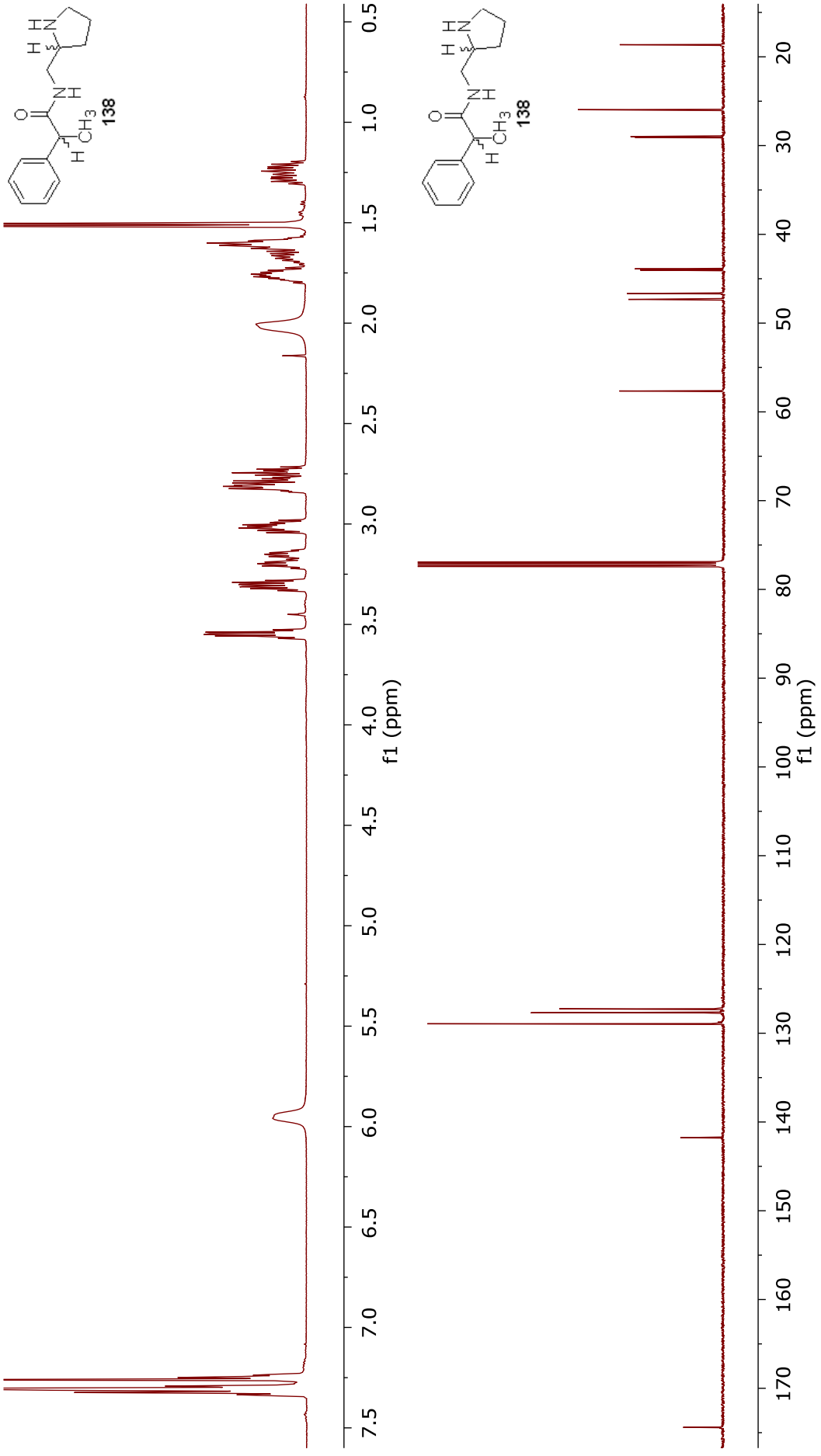

134
f1 (ppm)
134
f1 (ppm)

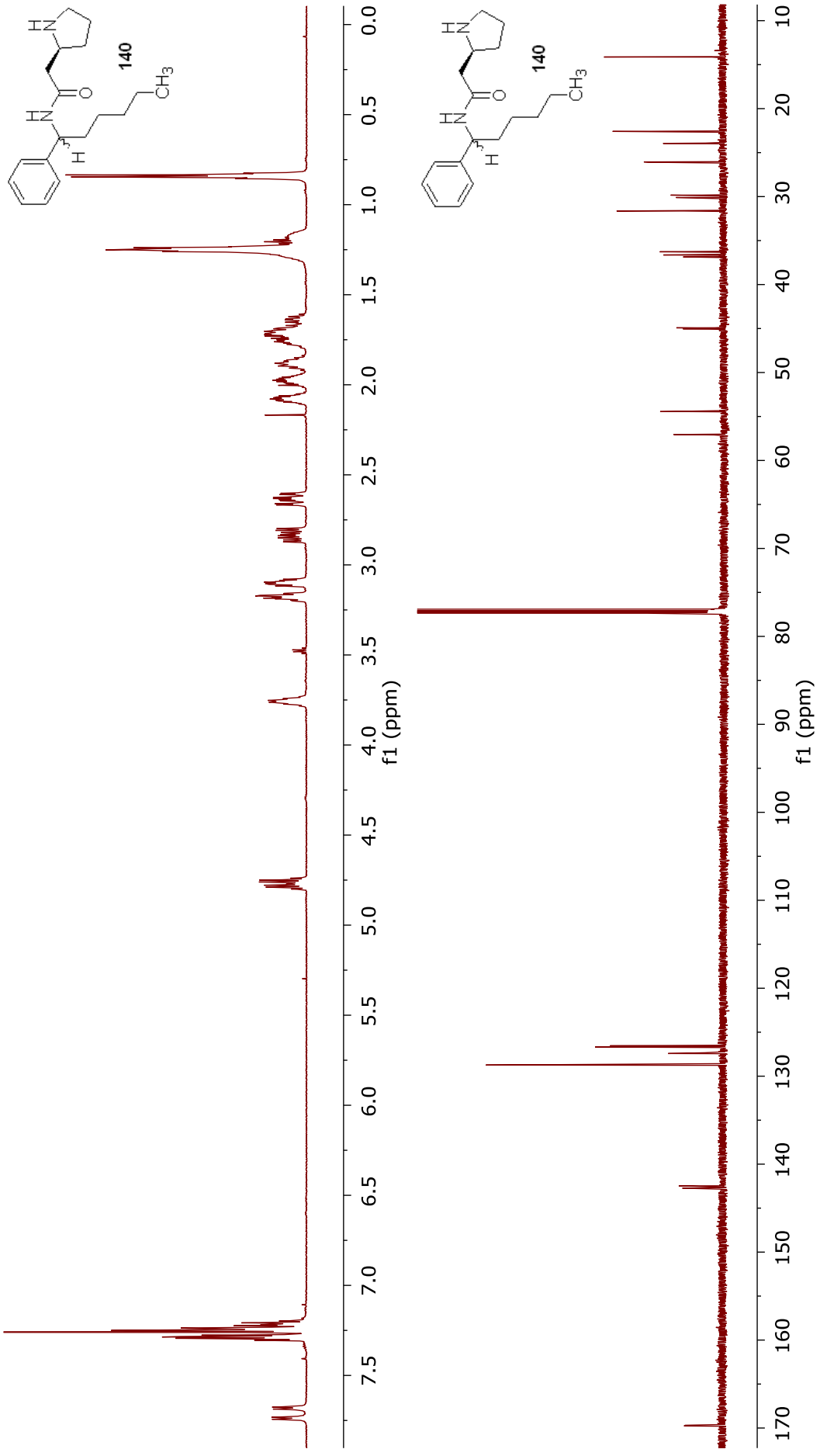

135
f1 (ppm)
135
f1 (ppm)

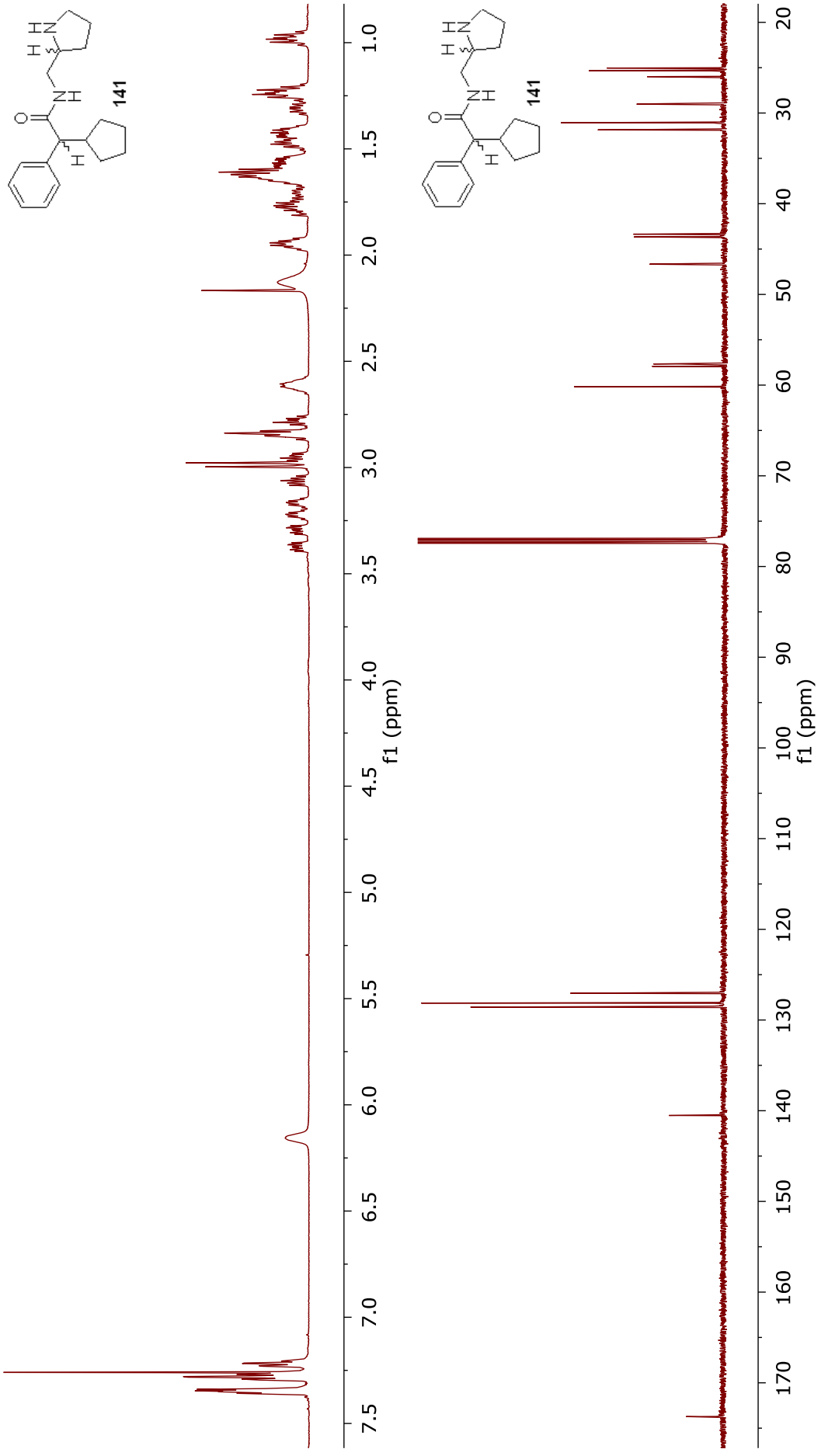


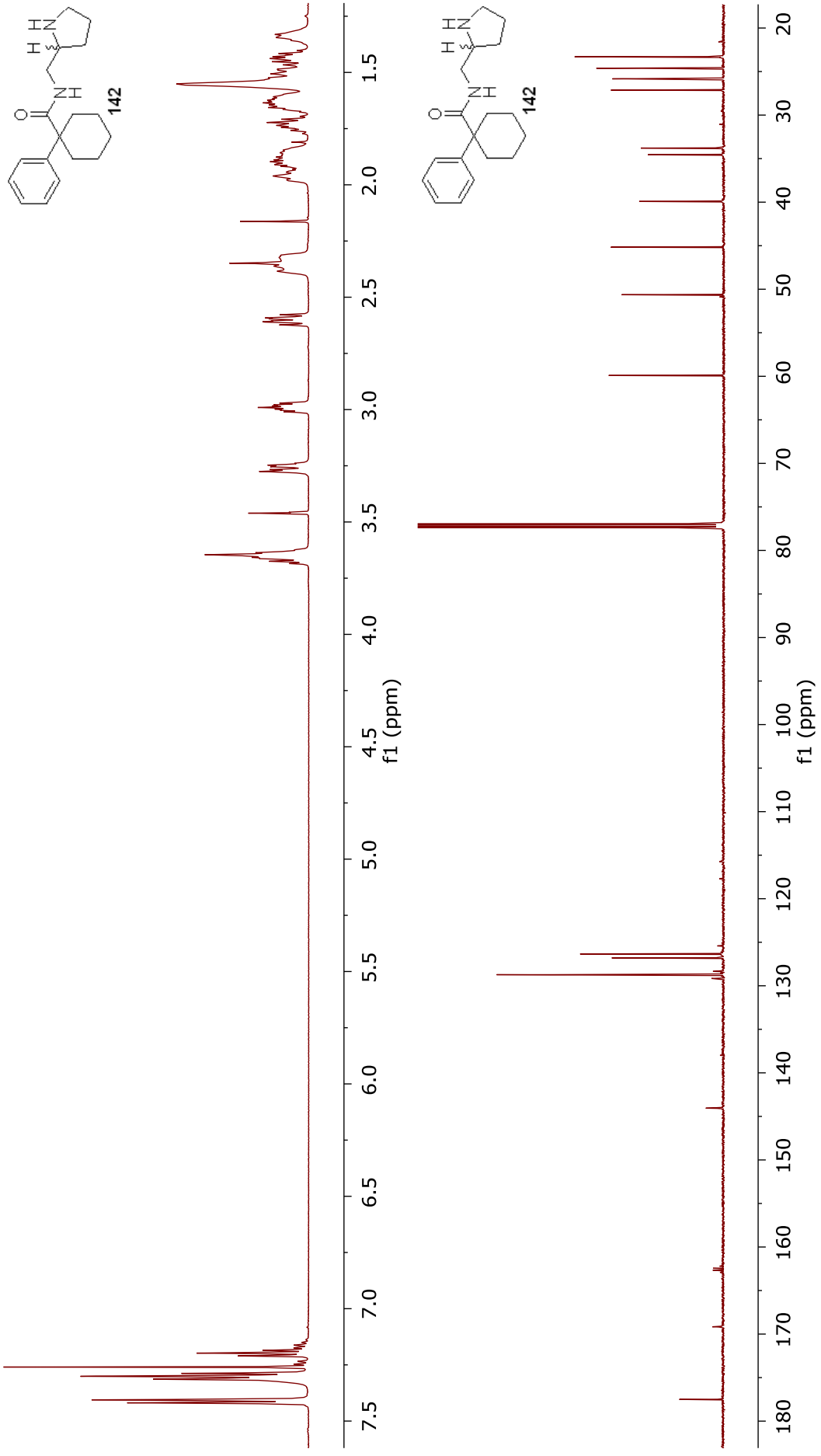
Note: Residual ether peaks at δ_H = 3.48 and 1.21 ppm; residual acetone peak at δ_H = 2.17 ppm

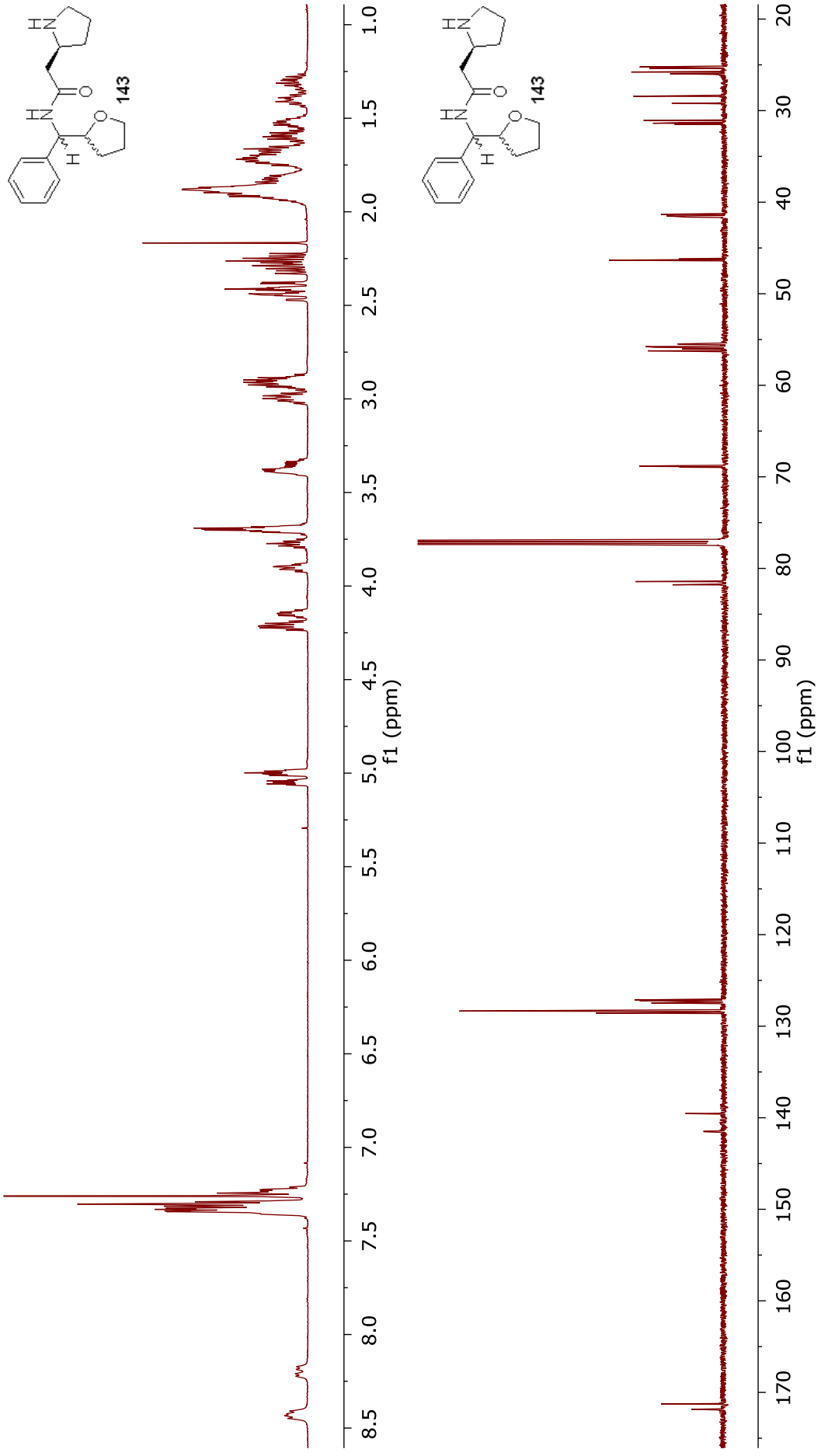

137
137
f1 (ppm)
f1 (ppm)

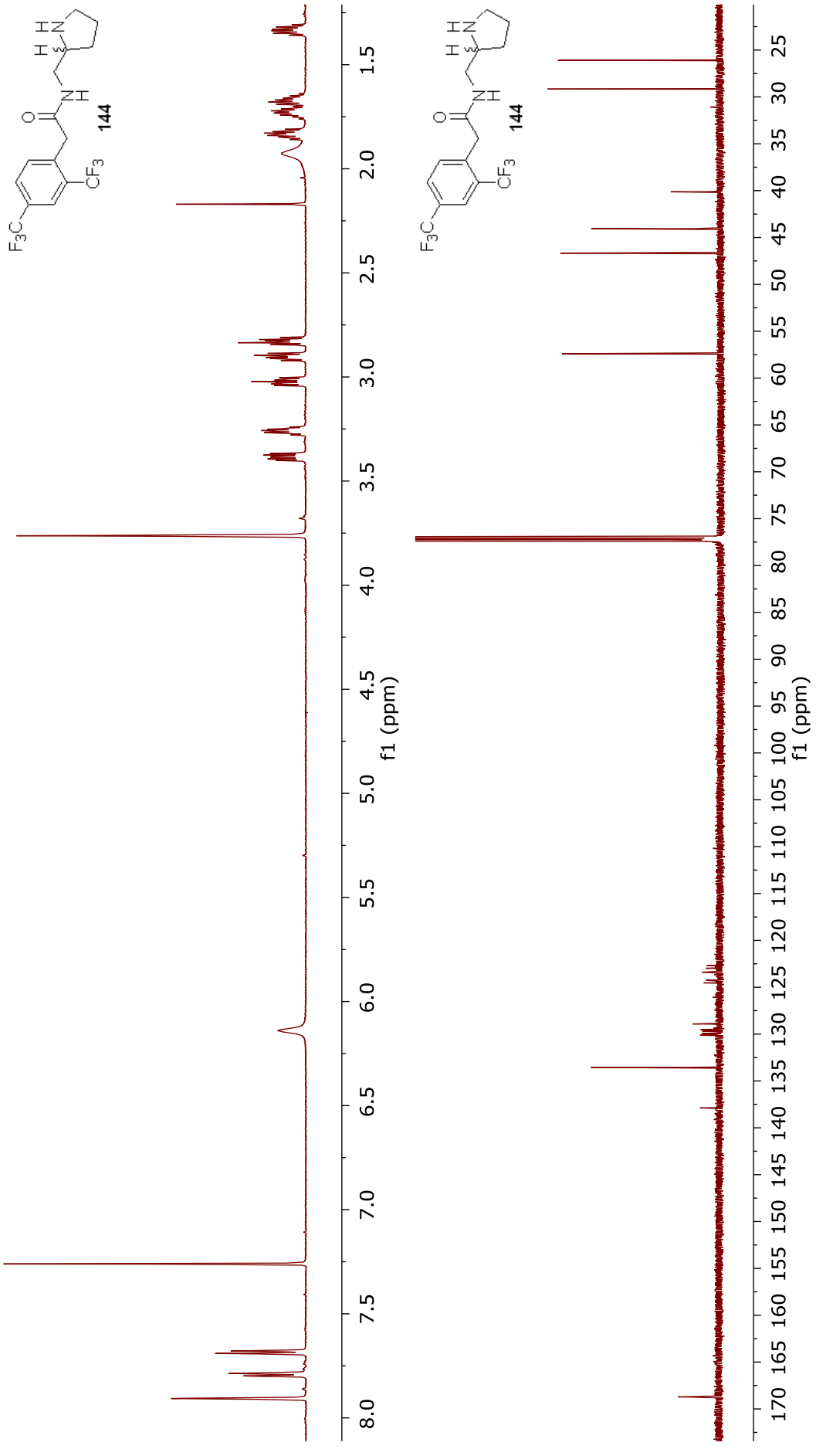

138
f1 (ppm)
138
f1 (ppm)

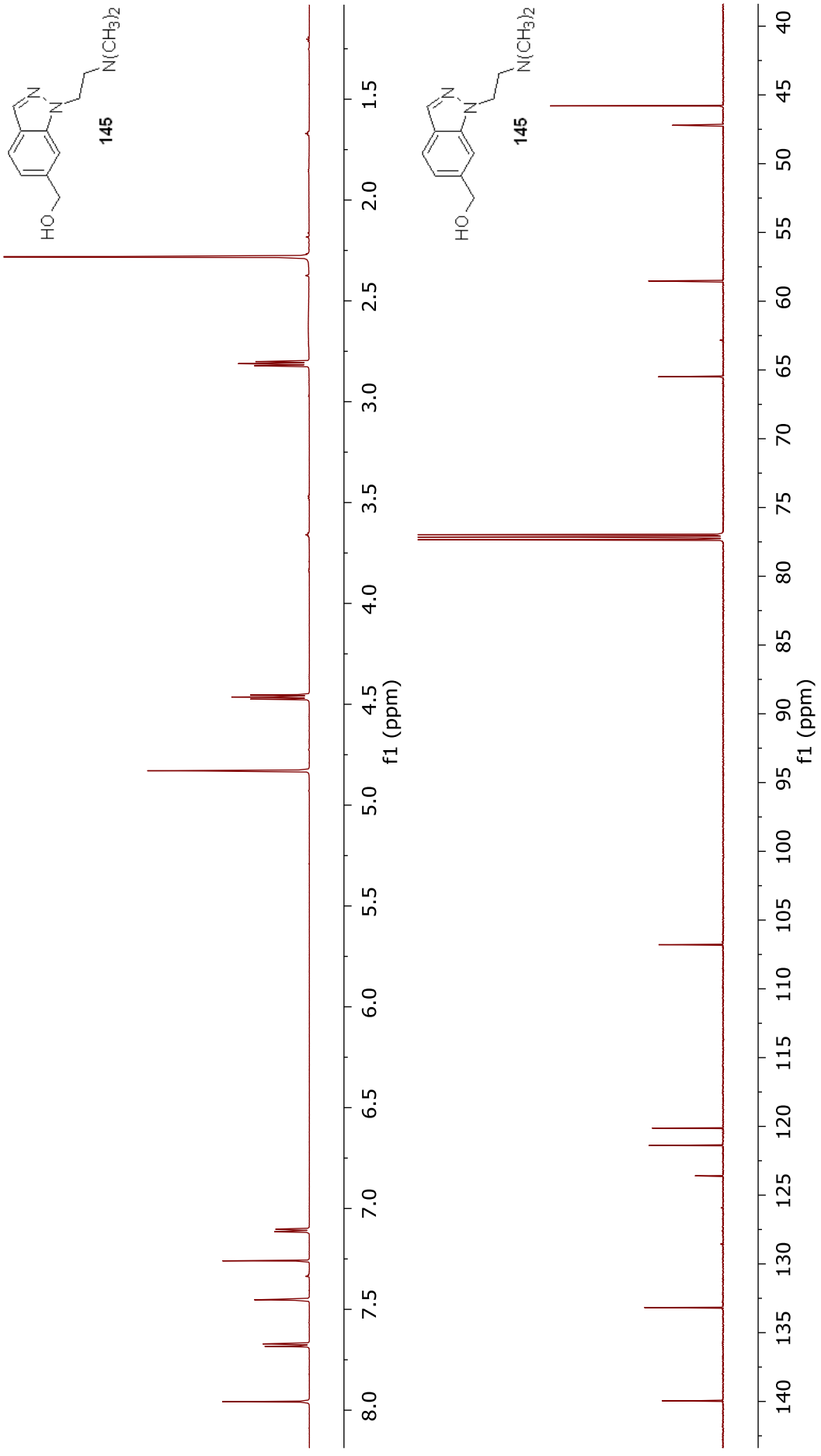

140
f1 (ppm)
140
f1 (ppm)

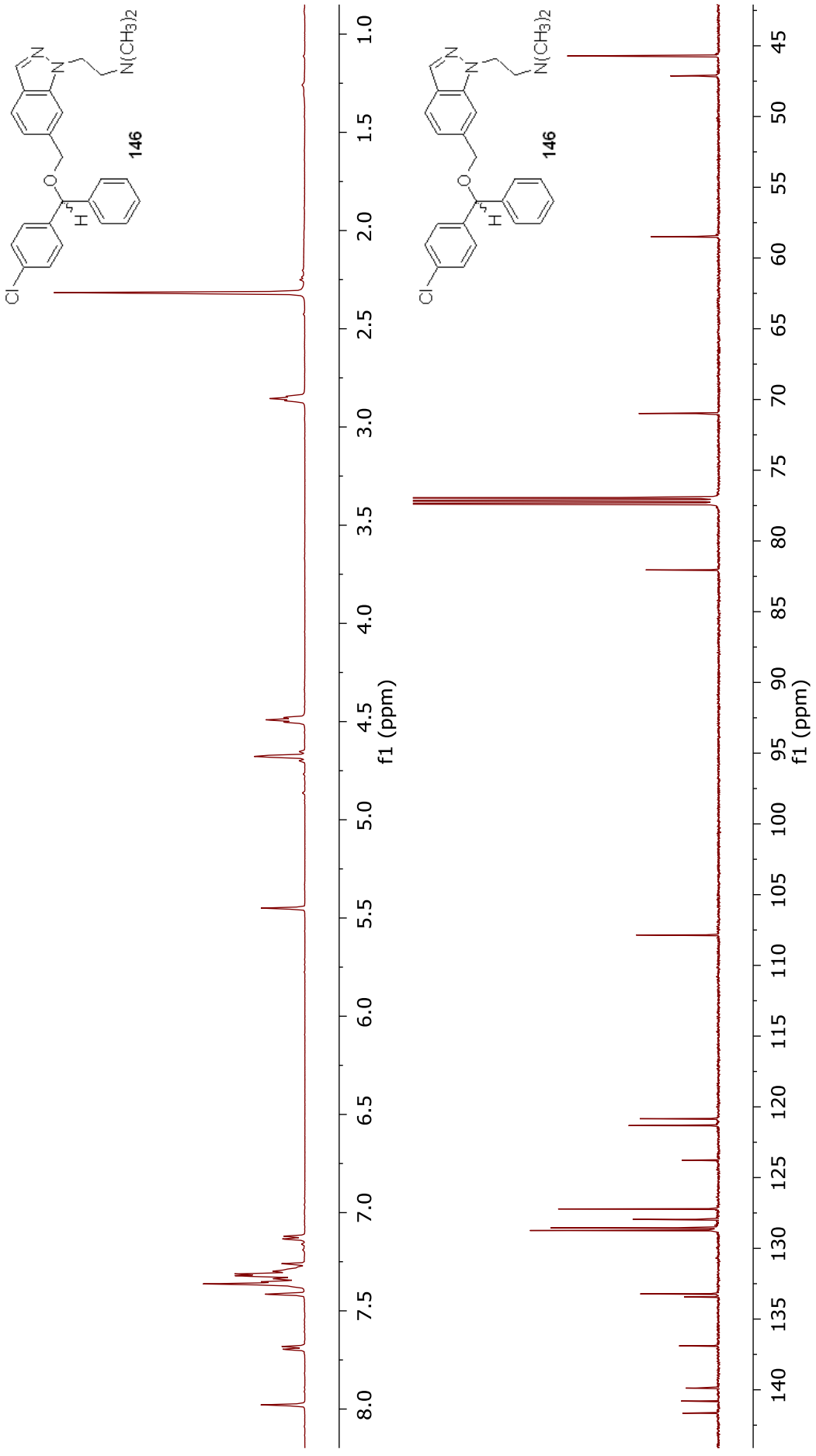

141
f1 (ppm)
141
f1 (ppm)

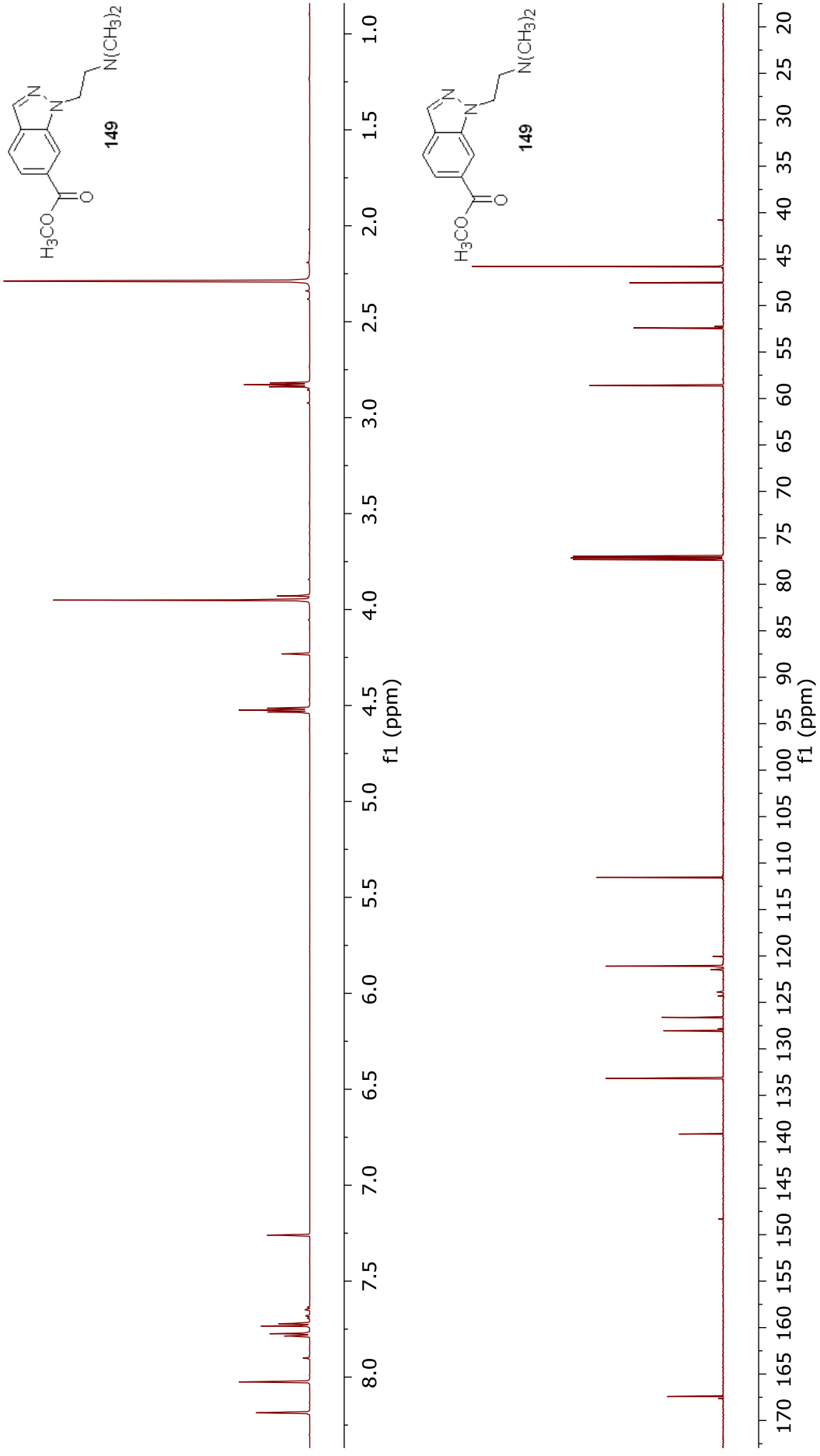

142
142
f1 (ppm)
f1 (ppm)

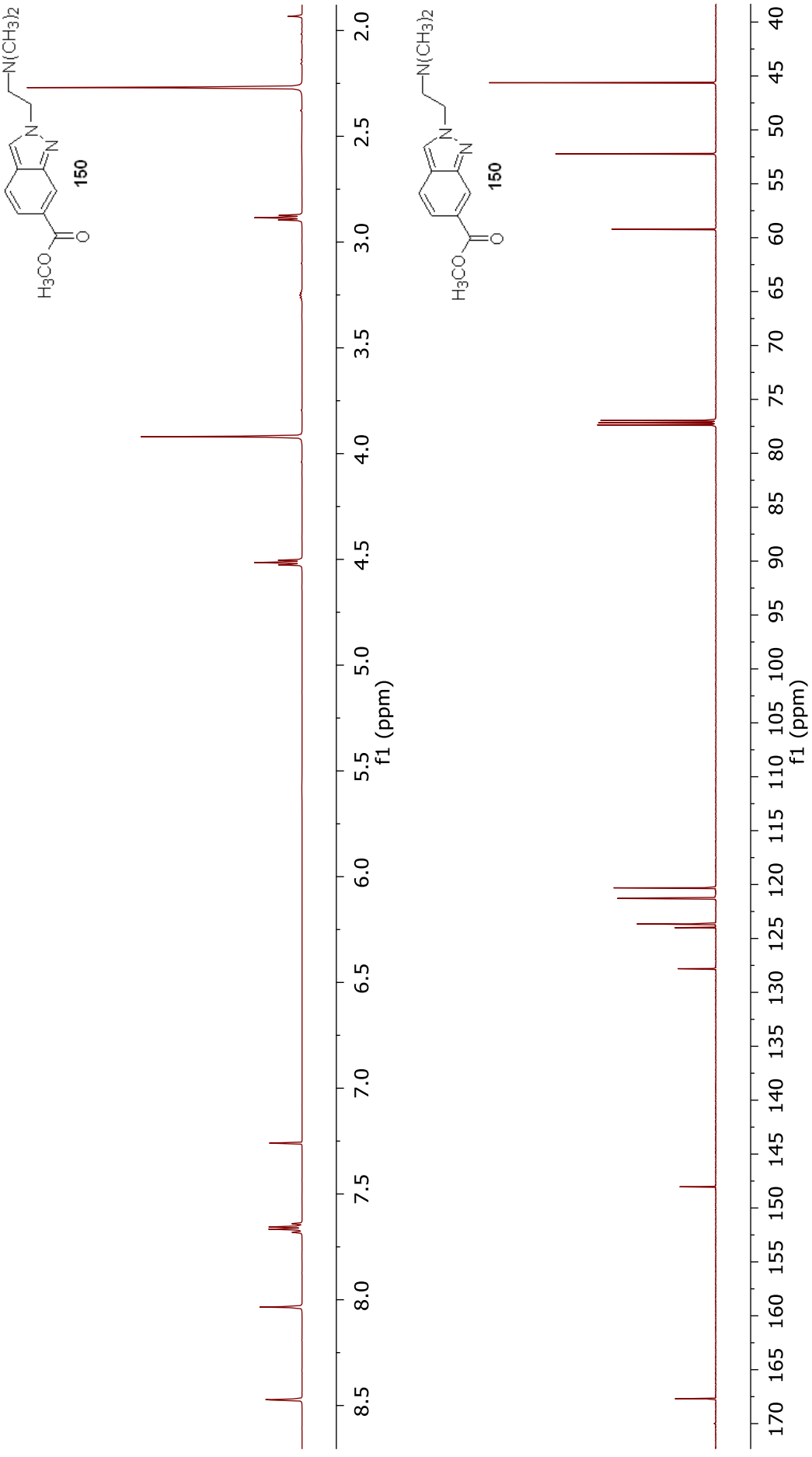

143
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)
143
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
f1 (ppm)

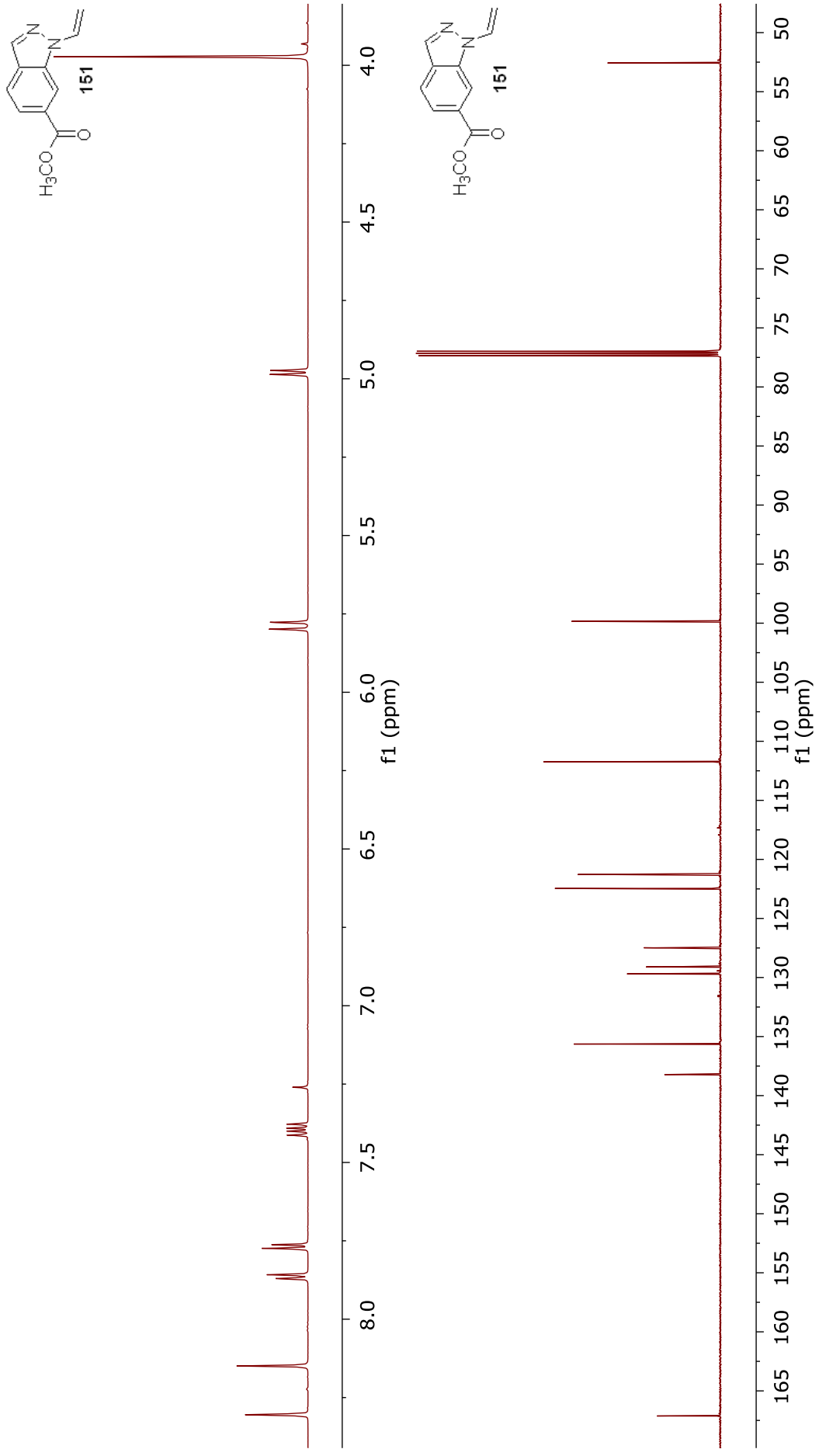

144
f1 (ppm)
144
f1 (ppm)

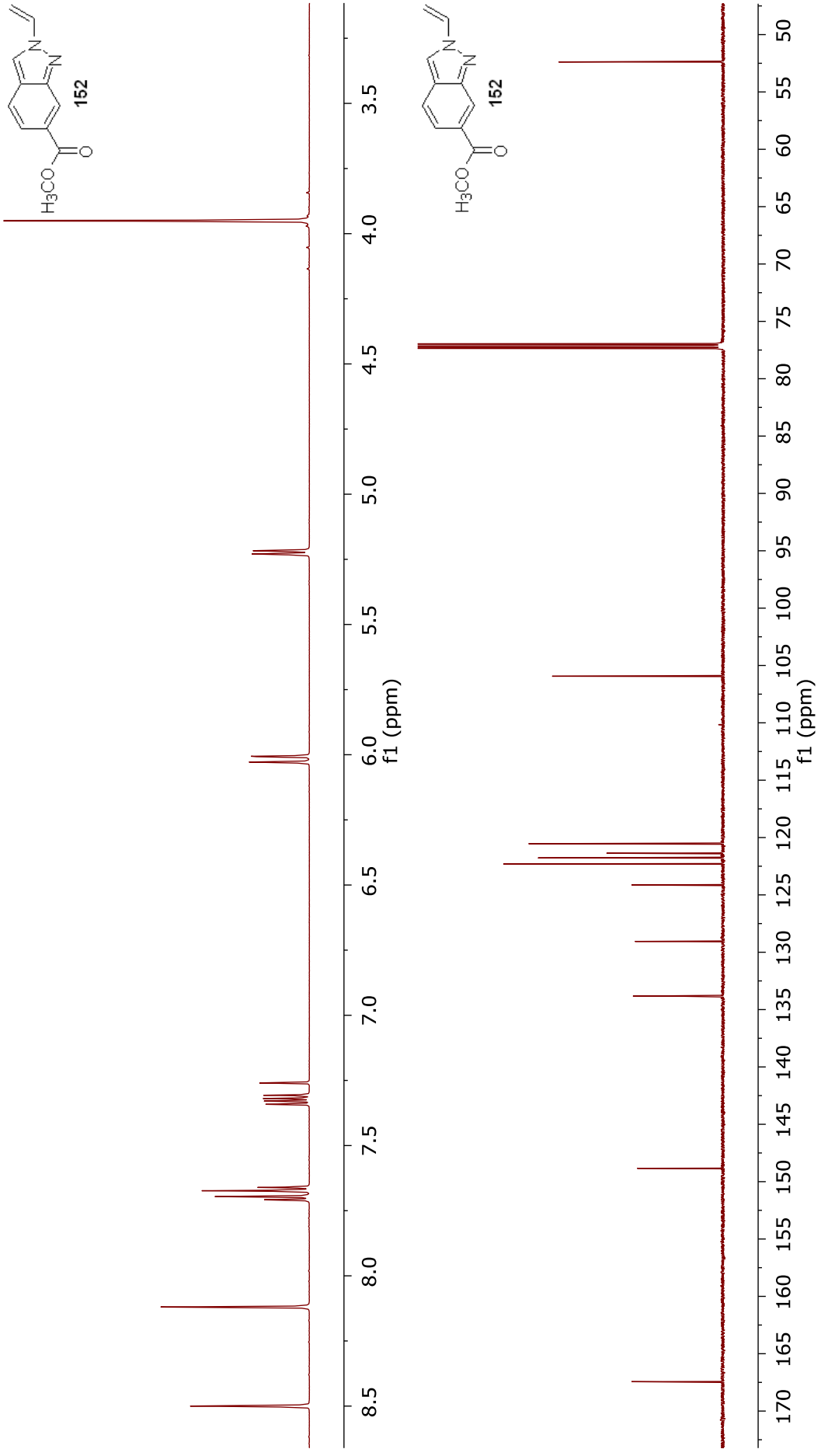

N(CH3)2
145
HO
f1 (ppm)
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
N(CH3)2
145
HO
f1 (ppm)
140
135
130
125
120
115
110
105
100
95
90
85
80
75
70
65
60
55
50
45
40

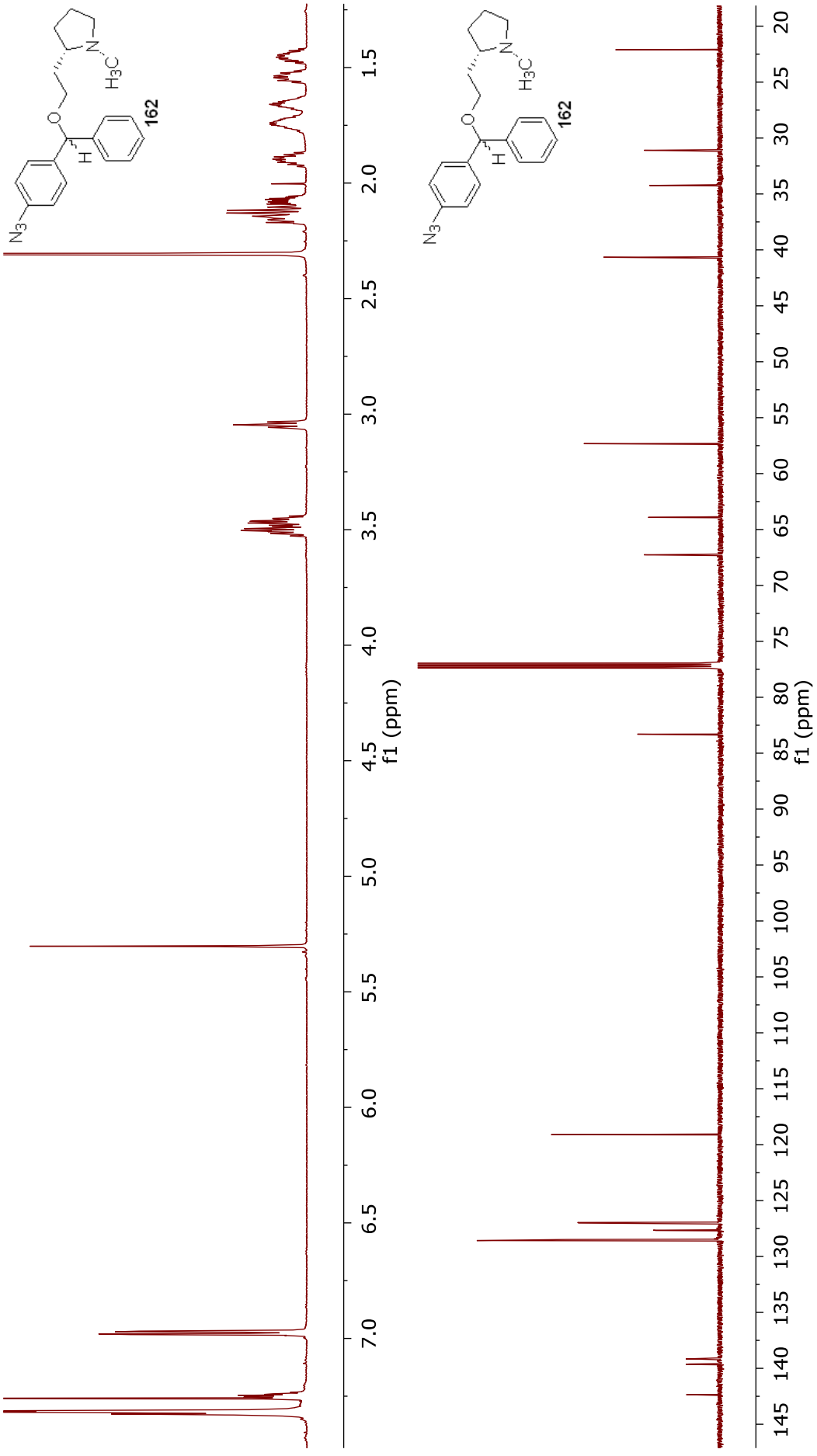

N(CH3)2
146
Cl
f1 (ppm)
N(CH3)2
146
Cl
f1 (ppm)

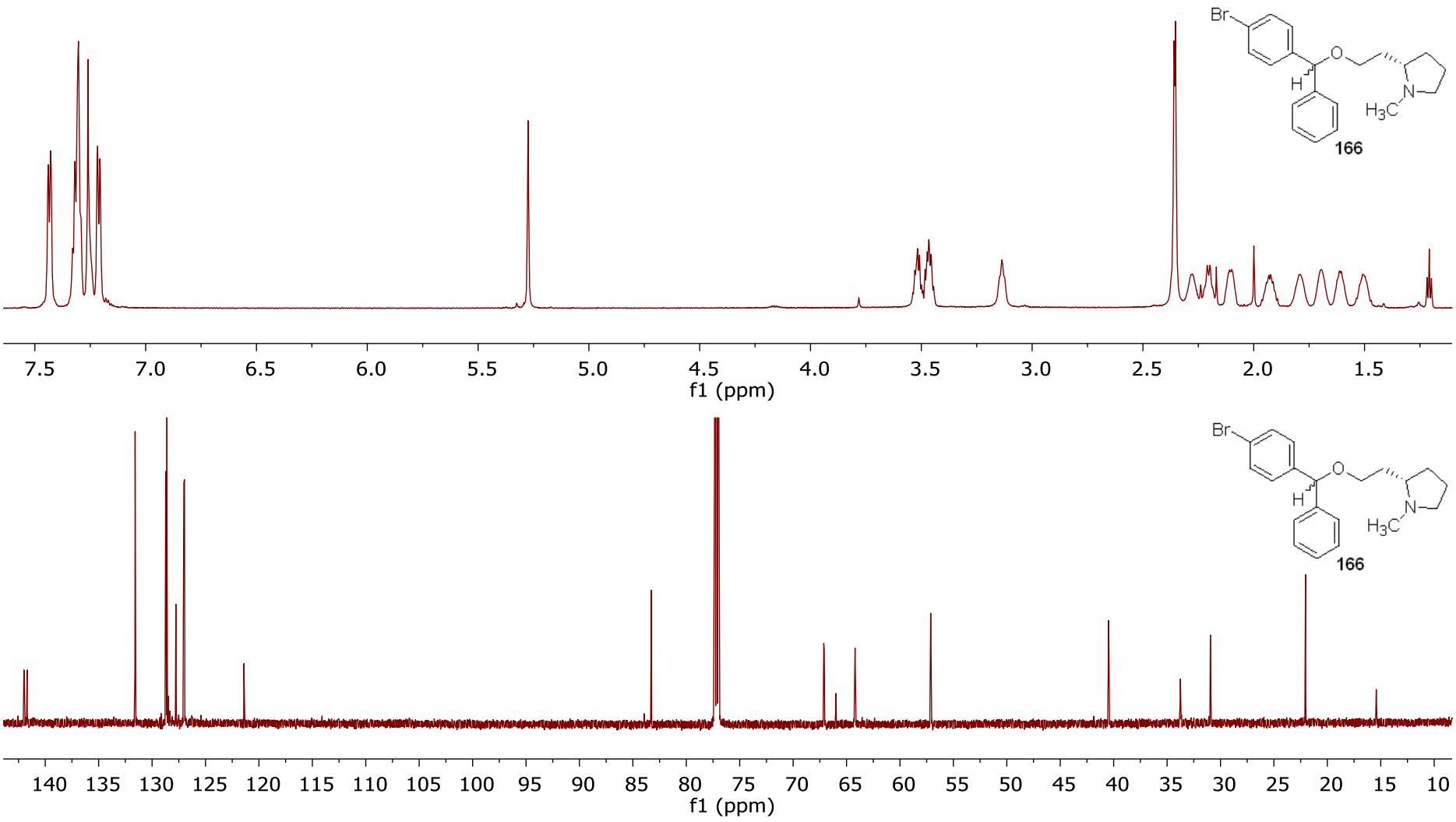

N(CH3)2
149
H3CO
O
f1 (ppm)
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0
N(CH3)2
149
H3CO
O
f1 (ppm)
170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20


N(CH3)2
N
N
150
H3CO
O
O
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f1 (ppm)
N(CH3)2
N
N
150
H3CO
O
O
170
165
160
155
150
145
140
135
130
125
120
115
110
105
100
95
90
85
80
75
70
65
60
55
50
45
40
f1 (ppm)

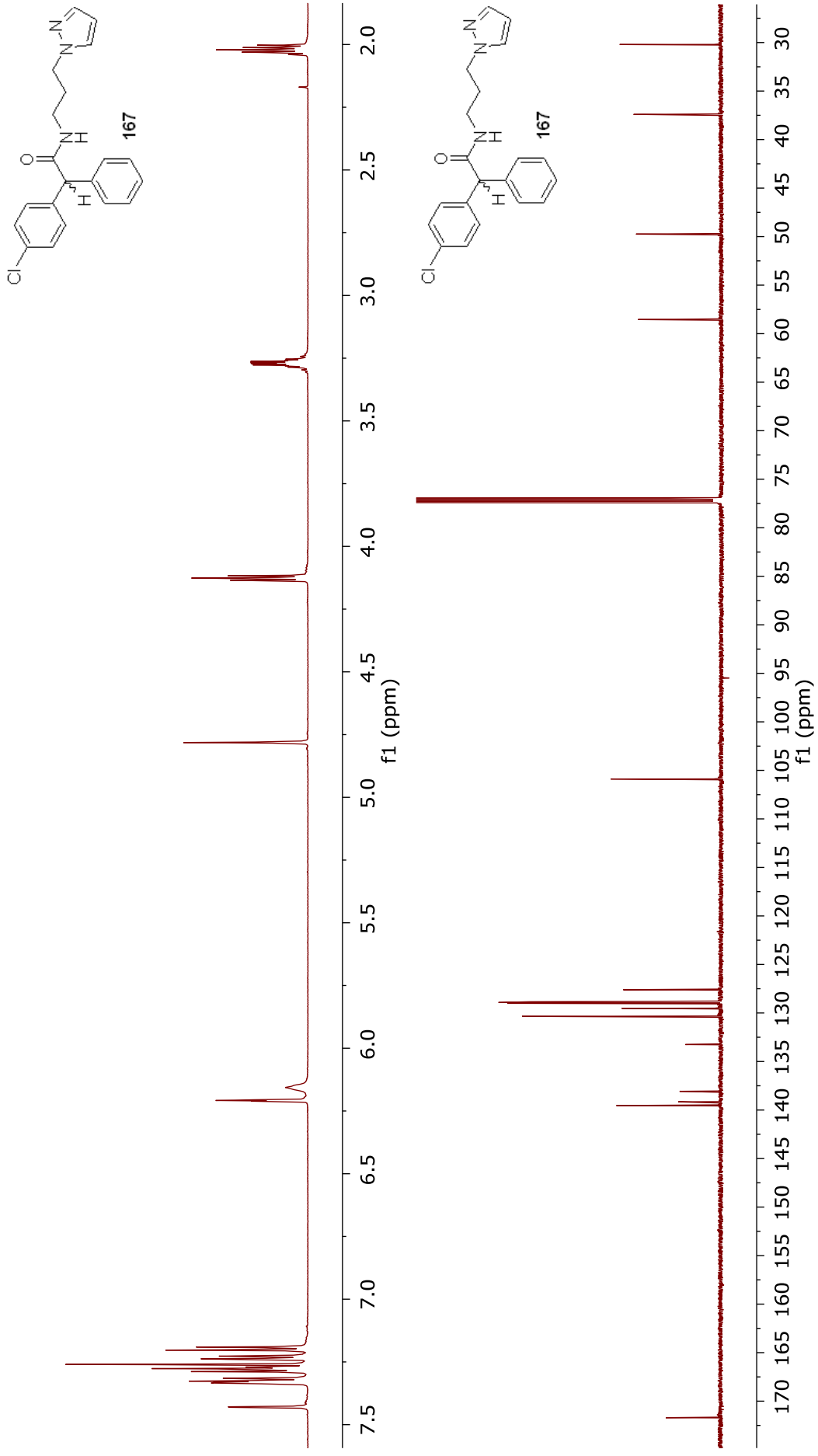

151
H3CO
O
O
N
N
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
f1 (ppm)
151
H3CO
O
O
N
N
165
160
155
150
145
140
135
130
125
120
115
110
105
100
95
90
85
80
75
70
65
60
55
50
f1 (ppm)

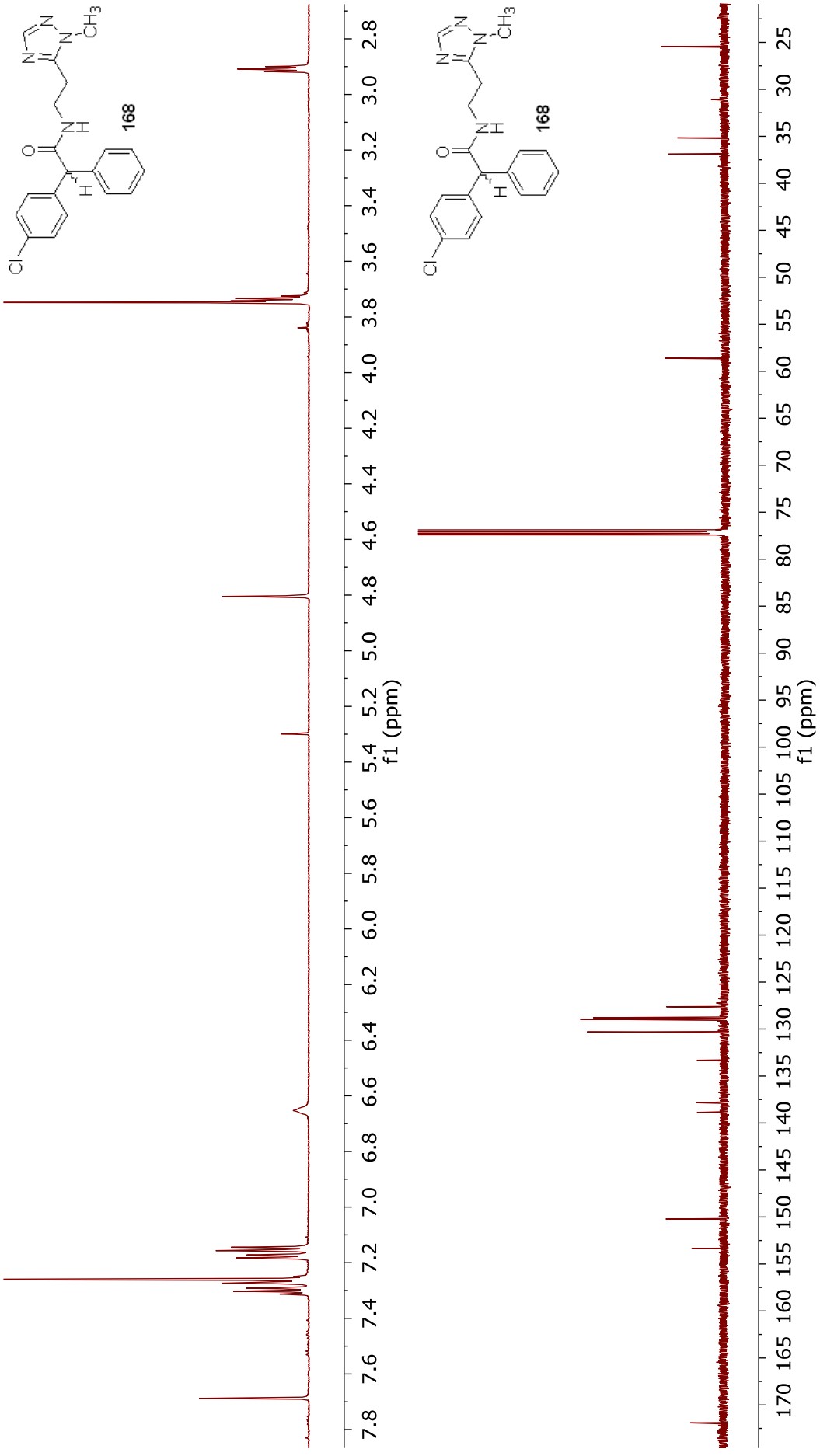

152
H3CO
O
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
f1 (ppm)
152
H3CO
O
170
165
160
155
150
145
140
135
130
125
120
115
110
105
100
95
90
85
80
75
70
65
60
55
50
f1 (ppm)

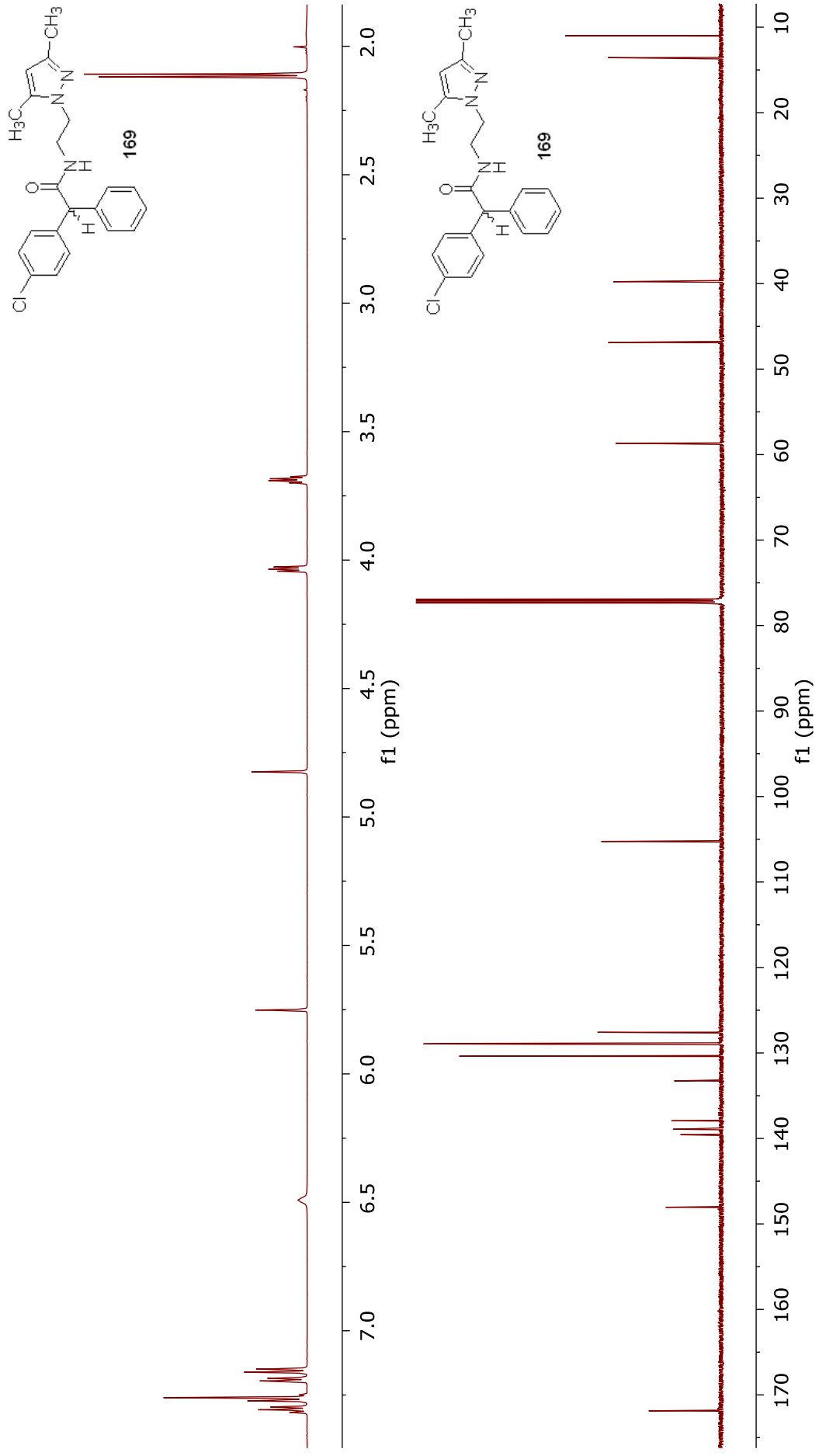

N3
H3C
N
O
H
162
f1 (ppm)
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
f1 (ppm)
145
140
135
130
125
120
115
110
105
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20

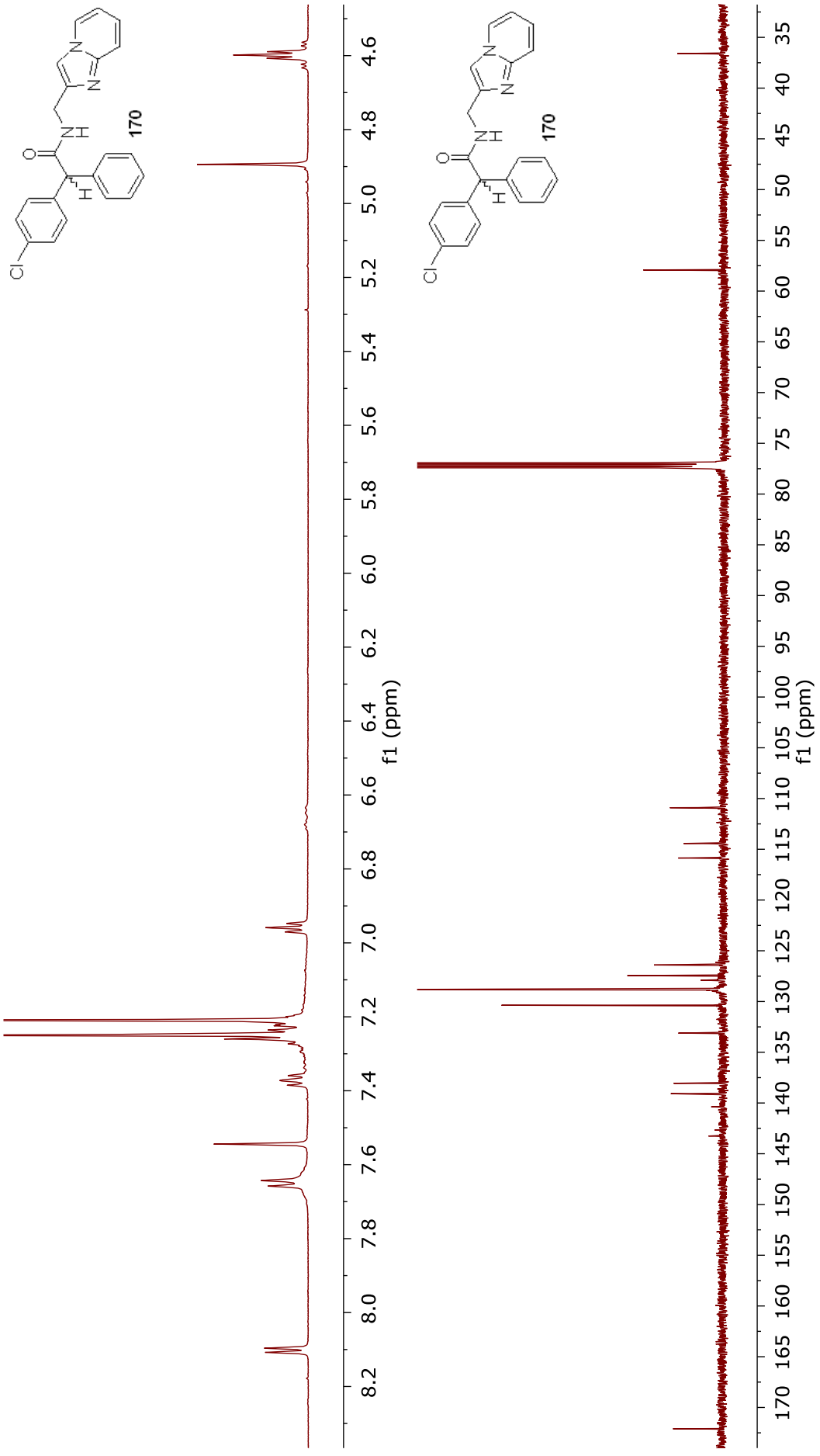


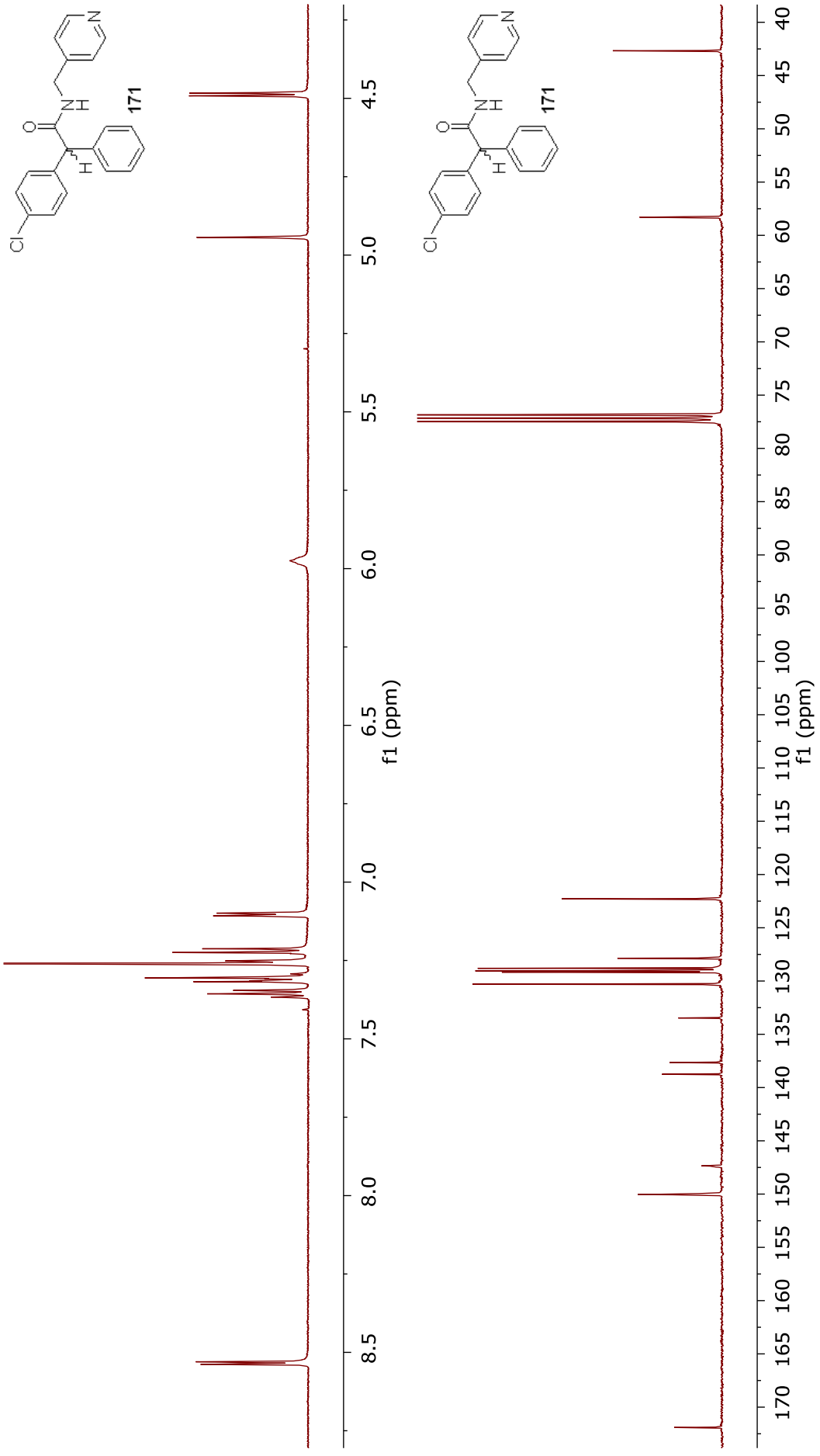
Note: Residual ether peaks observable at δ_H = 1.21 ppm; δ_C = 66.0 and 15.4 ppm

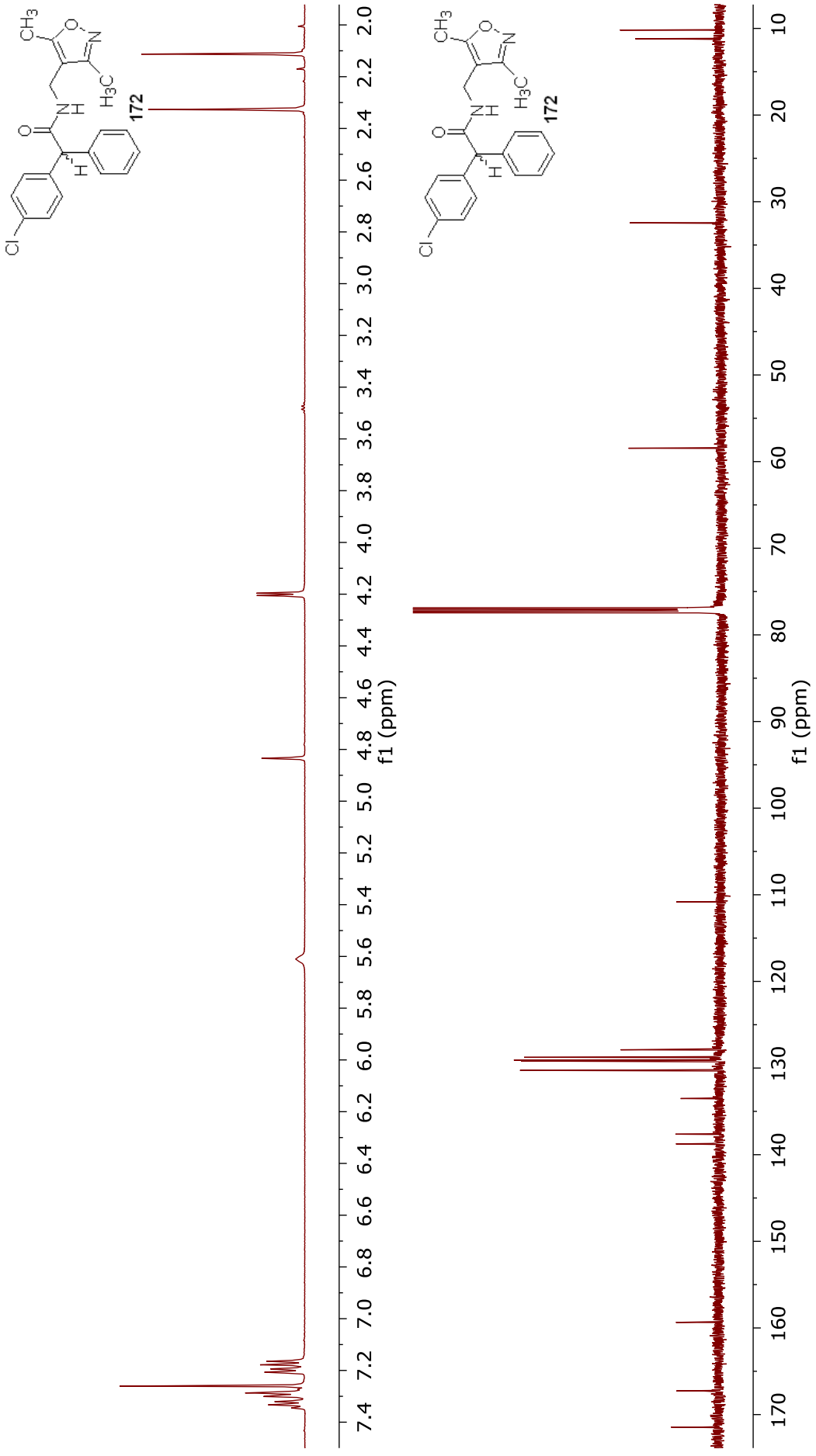

167
f1 (ppm)
167
f1 (ppm)

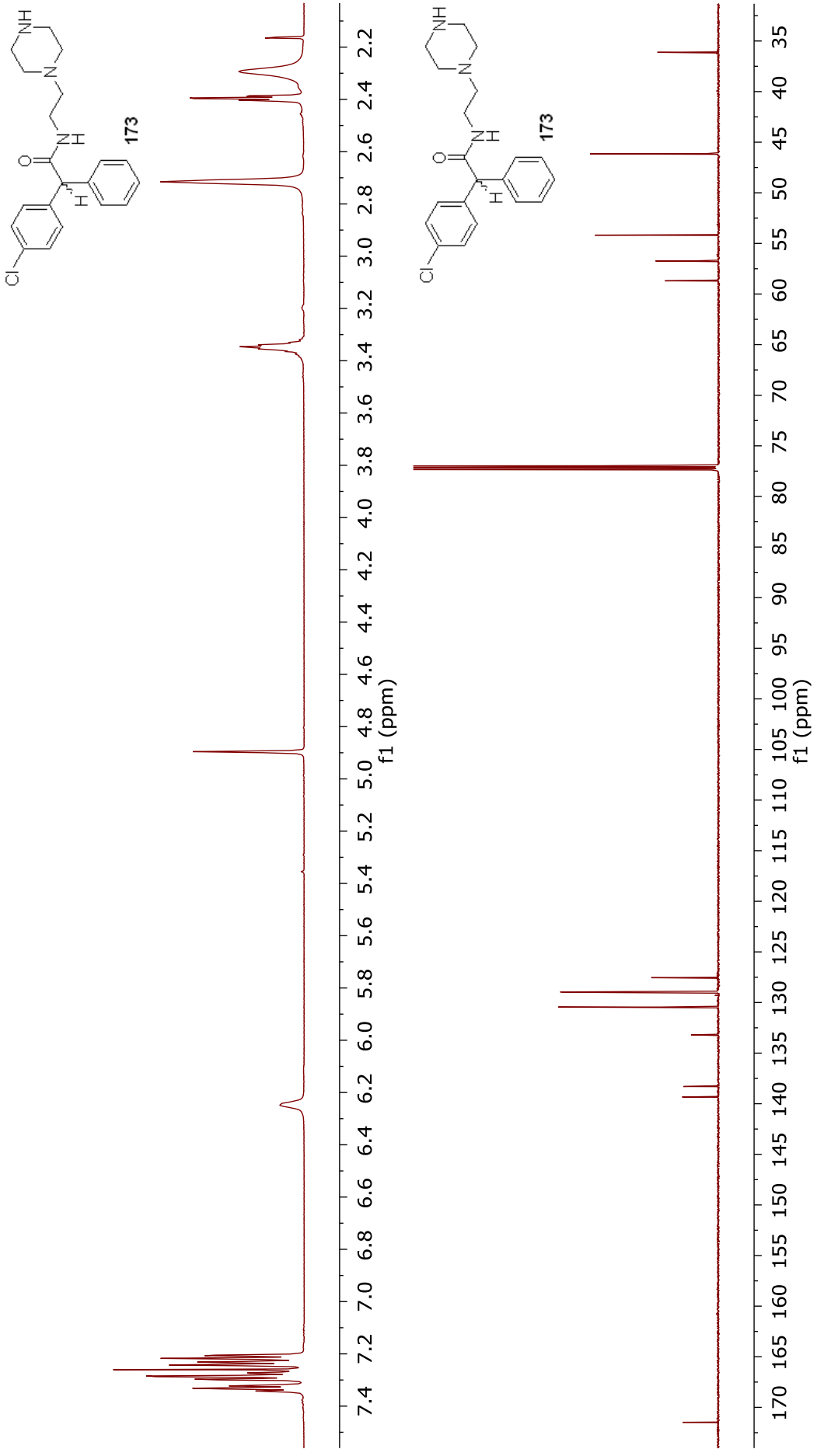

168
f1 (ppm)
168
f1 (ppm)

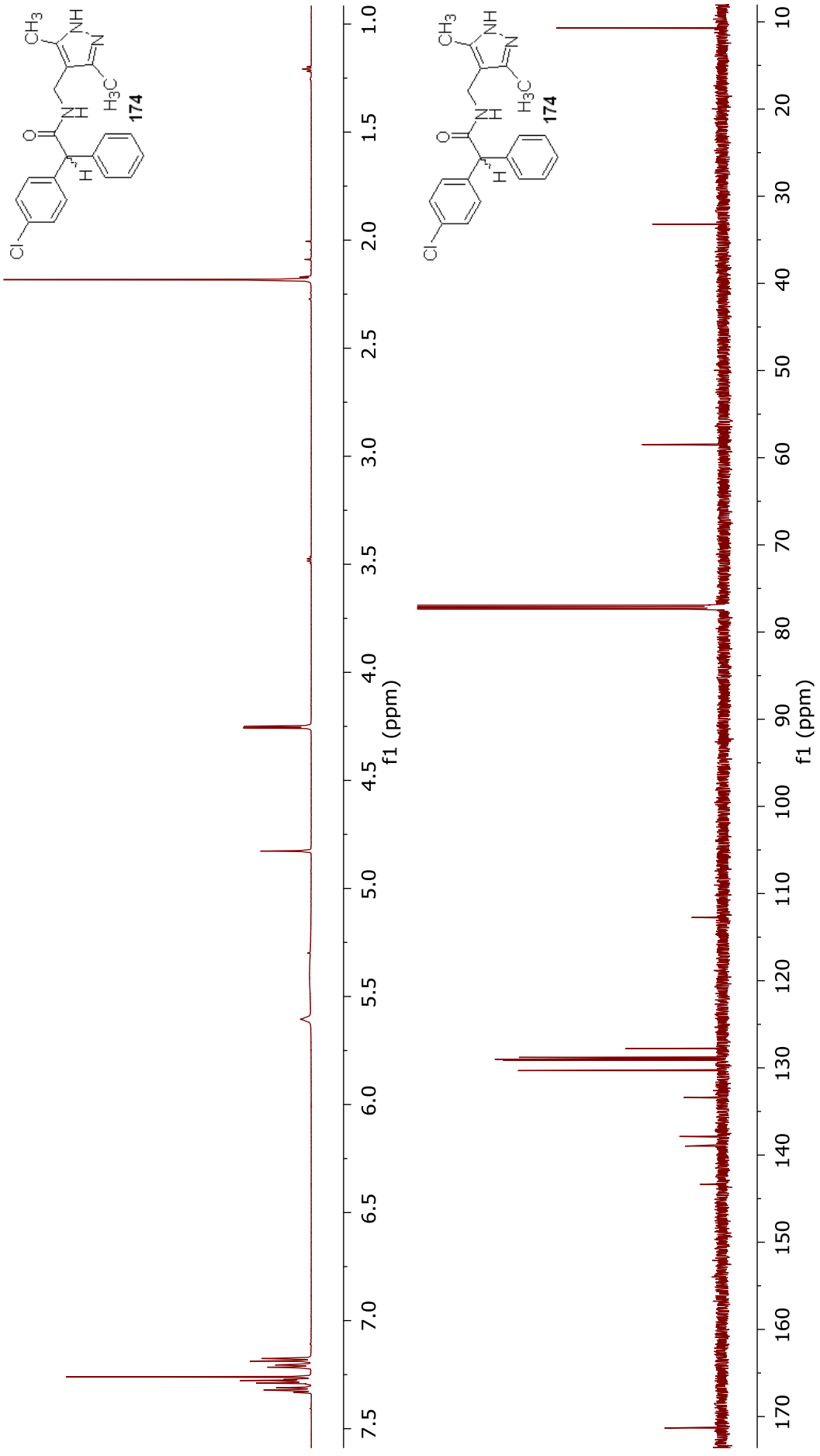

169
f1 (ppm)
169
f1 (ppm)

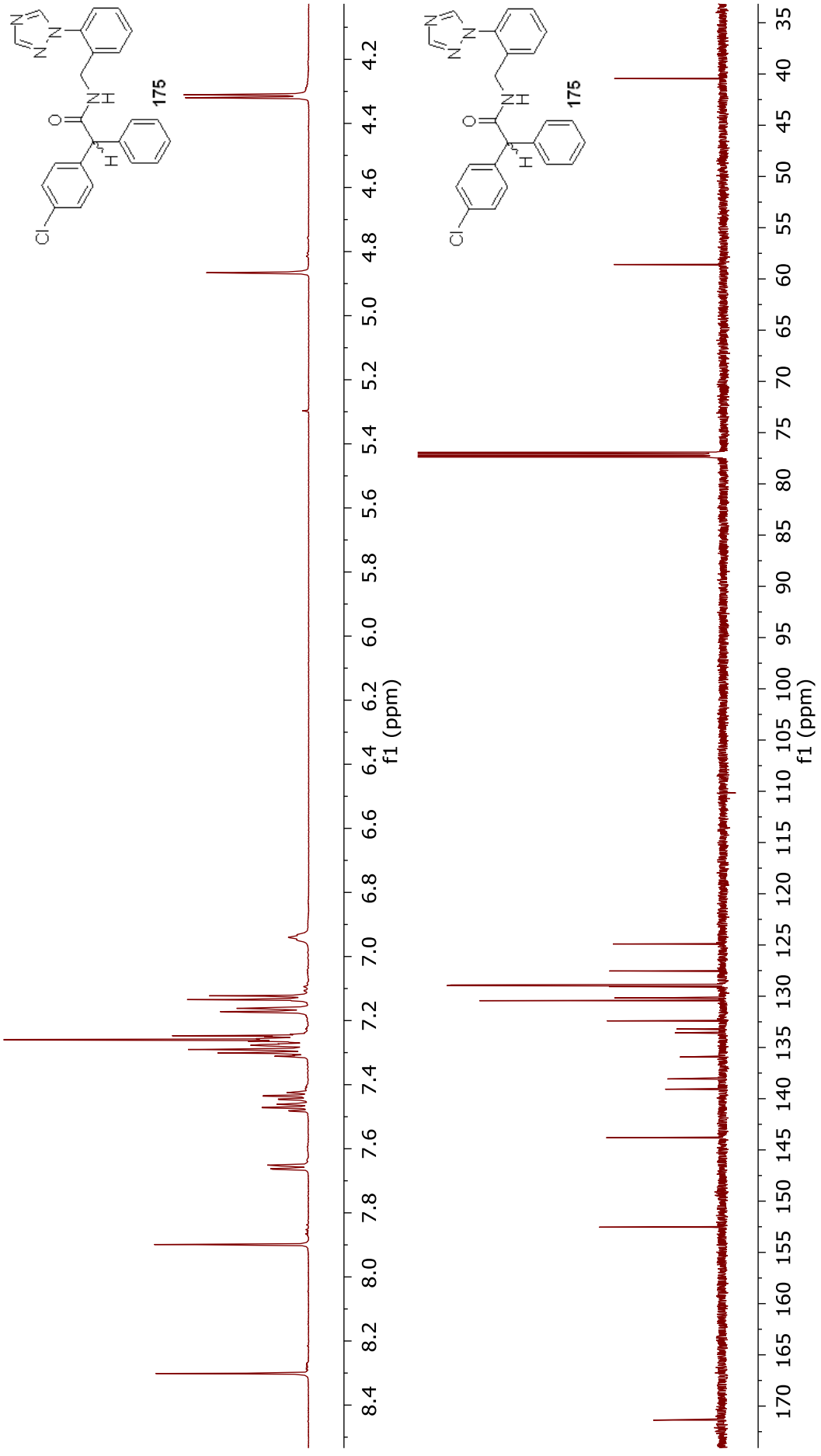

170
f1 (ppm)
170
f1 (ppm)

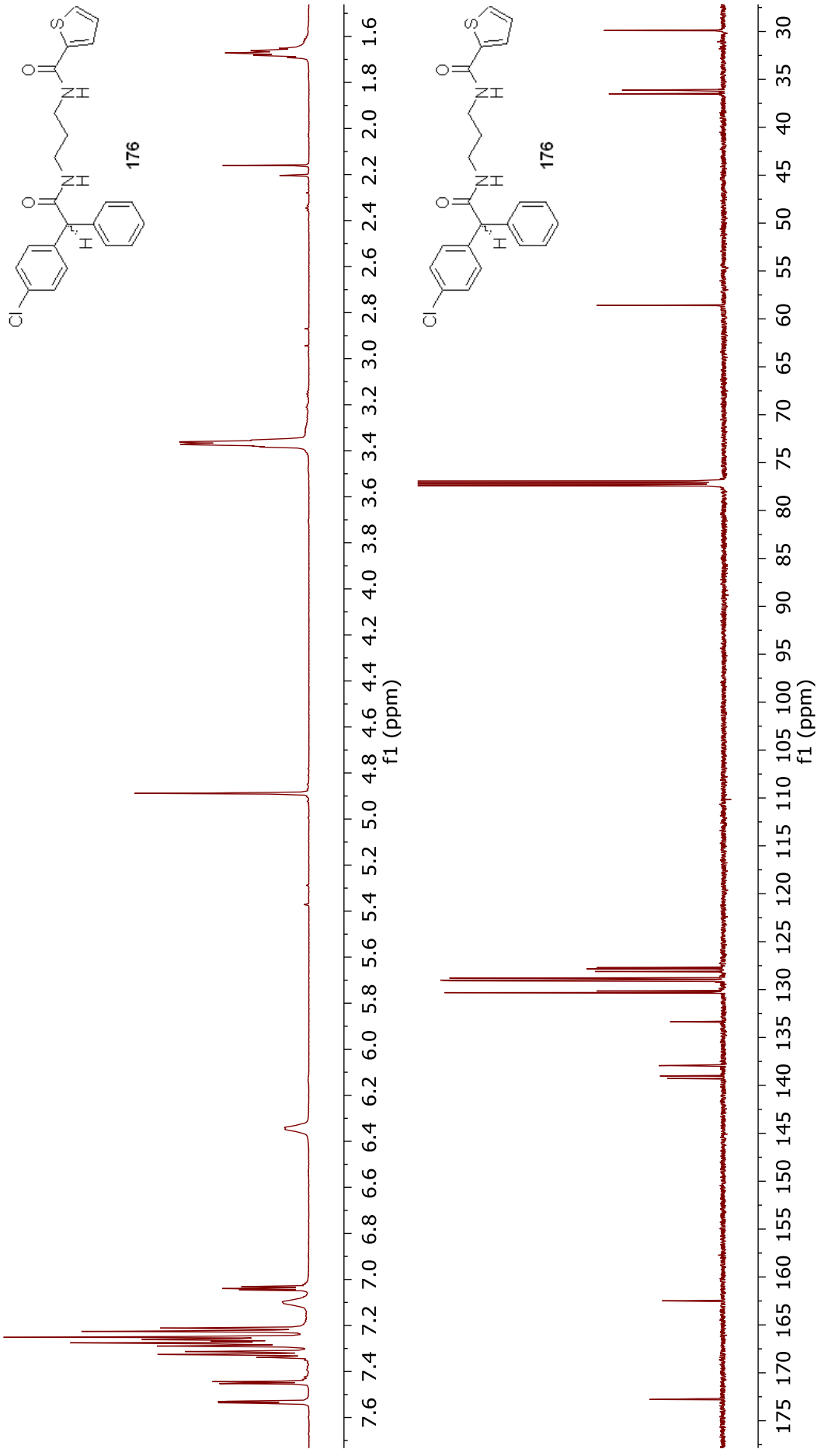

171
171
f1 (ppm)
f1 (ppm)

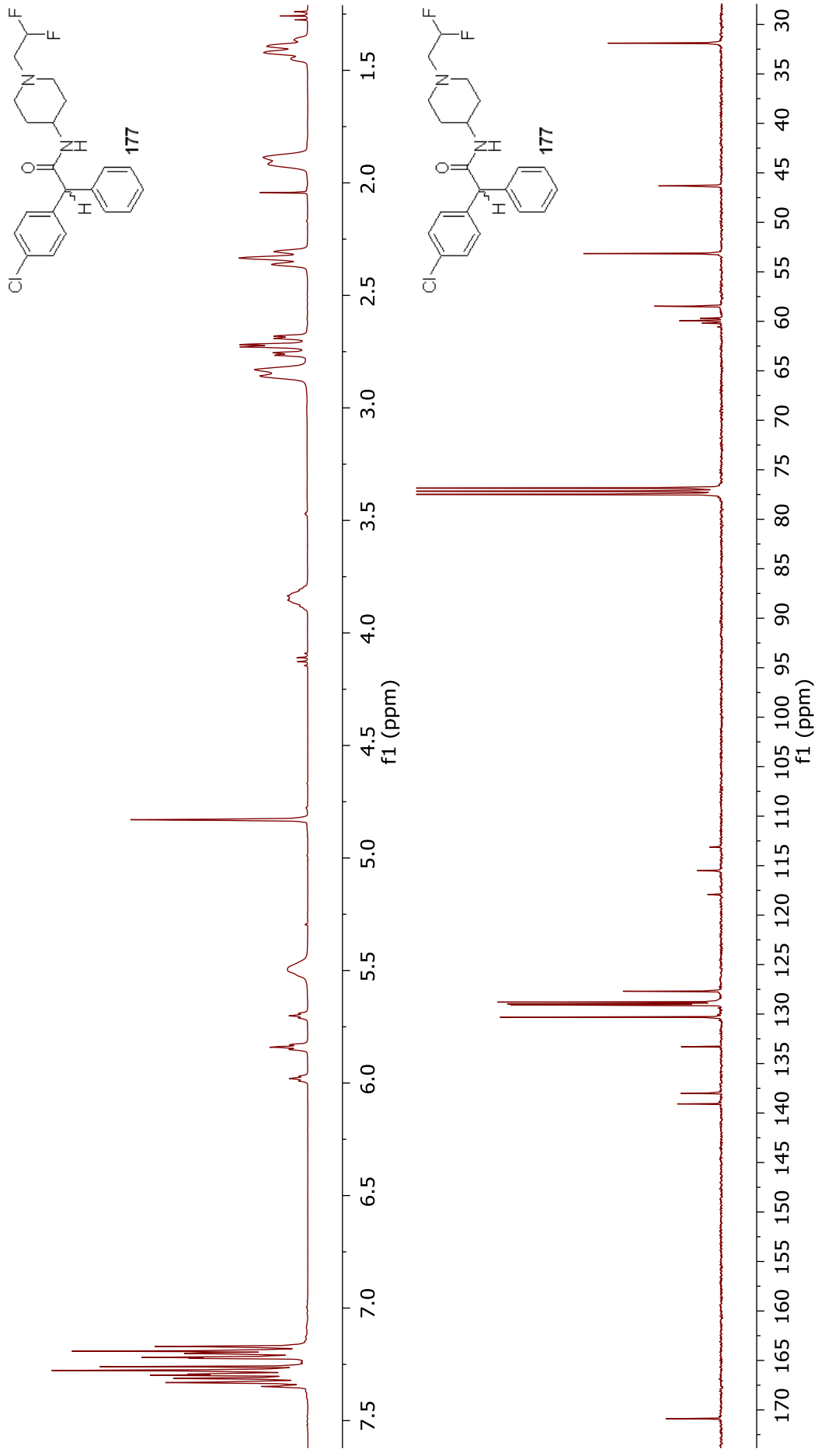

CH3
O
N
H3C
N
H
O
H
Cl
172
f1 (ppm)
f1 (ppm)

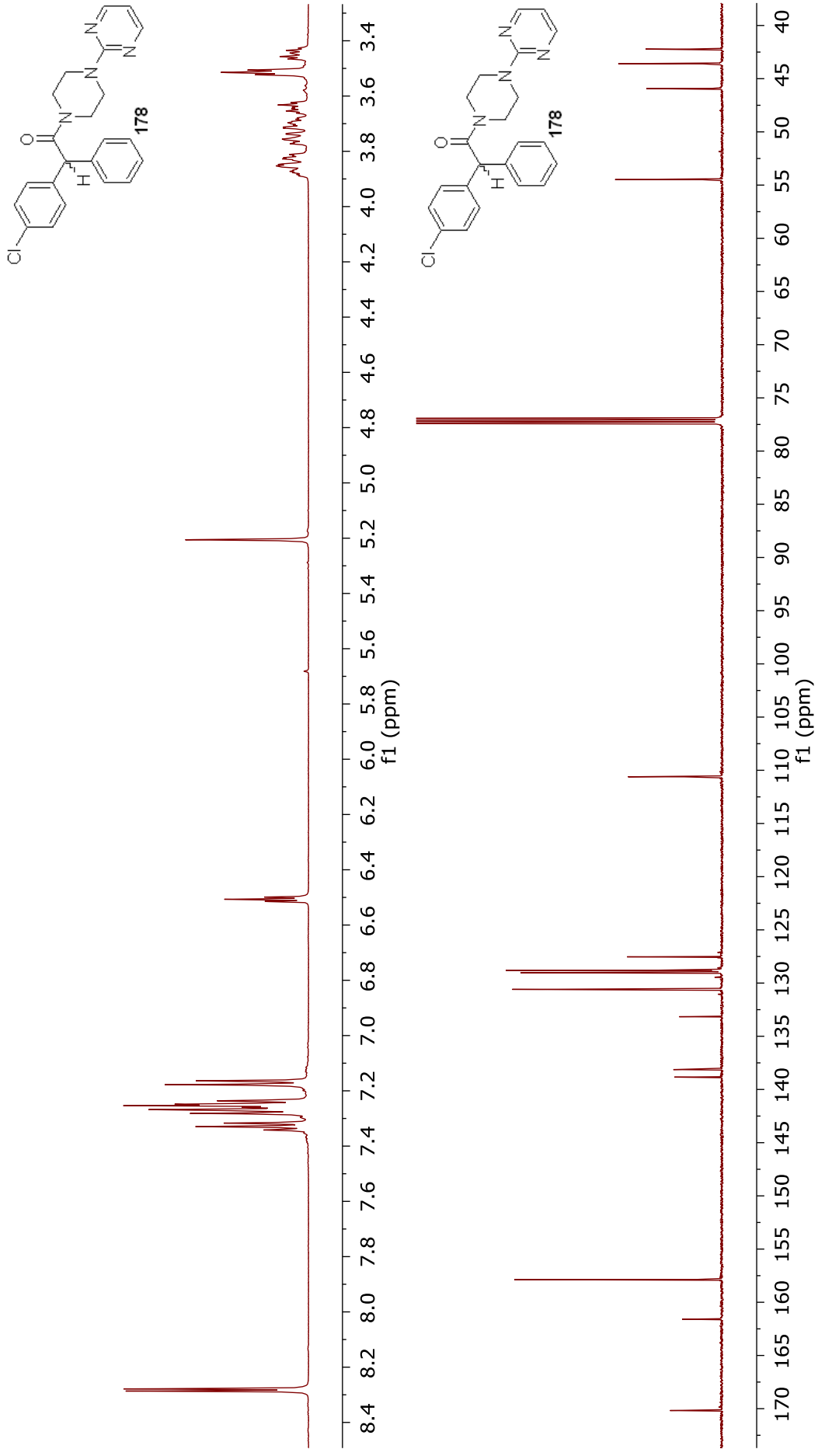

NH
N
NH
O
H
Cl
173
f1 (ppm)
NH
N
NH
O
H
Cl
173
f1 (ppm)

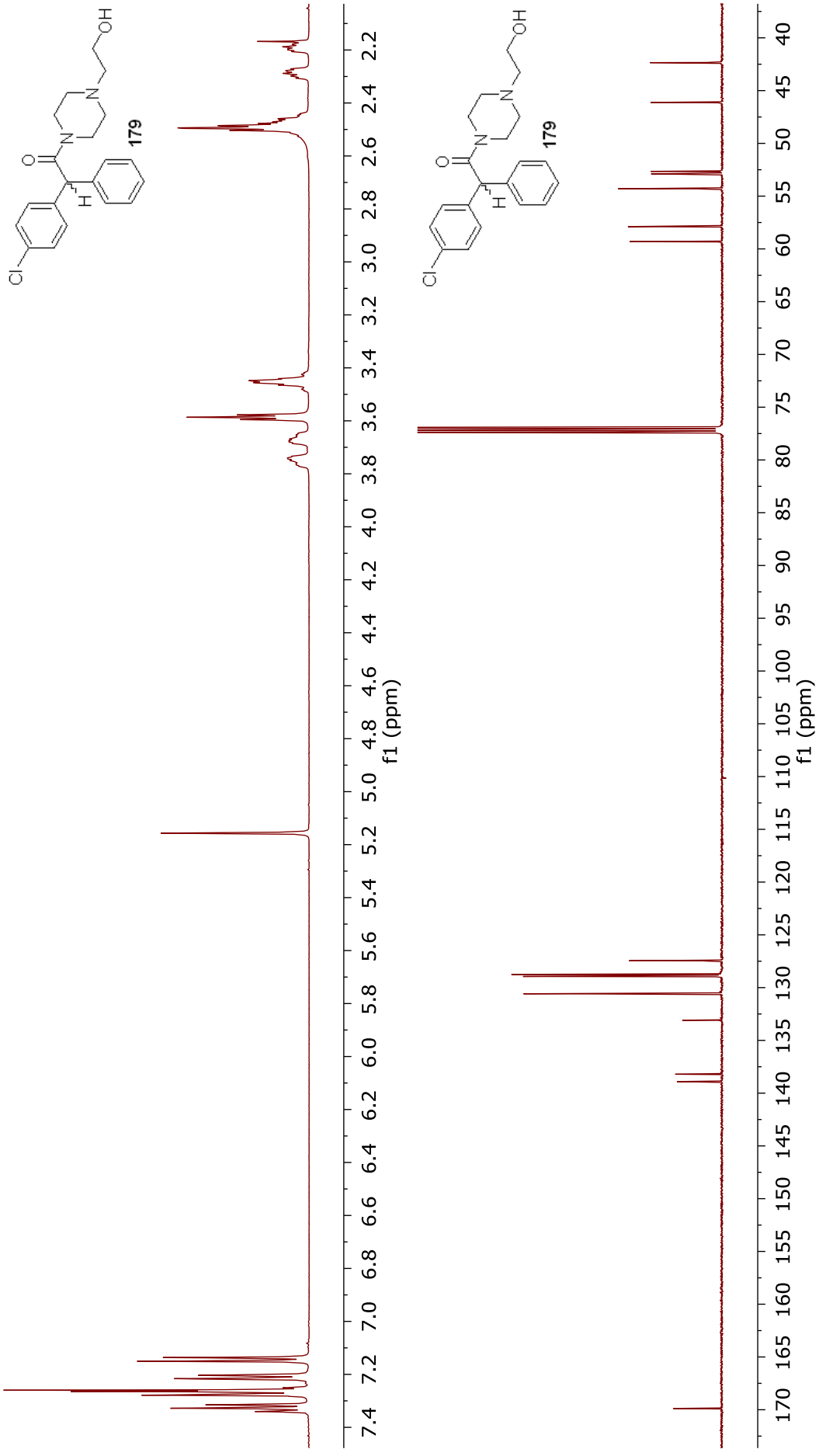

CH3
NH
N
H3C
O
N
H
H
Cl
174
f1 (ppm)
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170

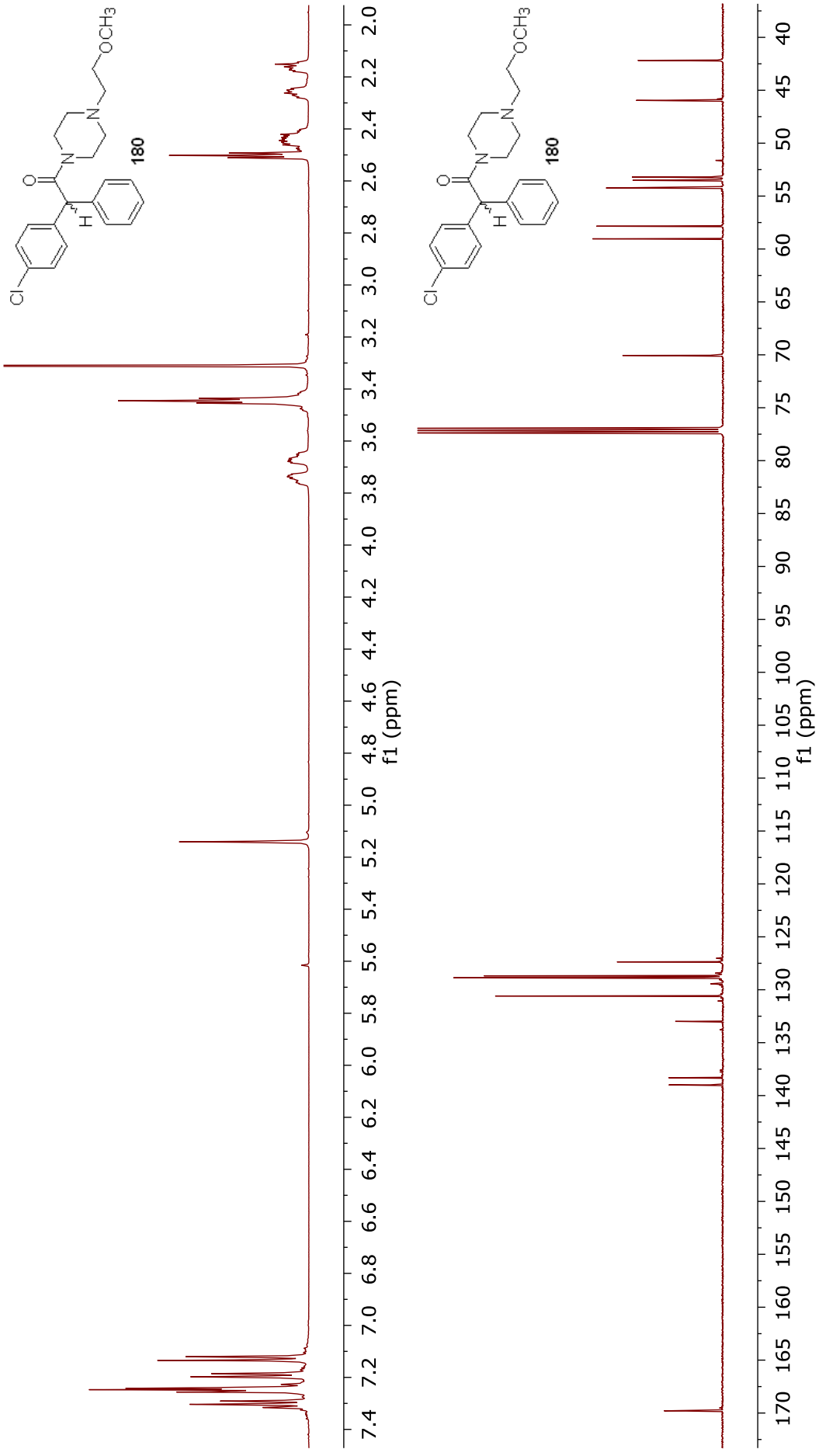

175
f1 (ppm)
175
f1 (ppm)

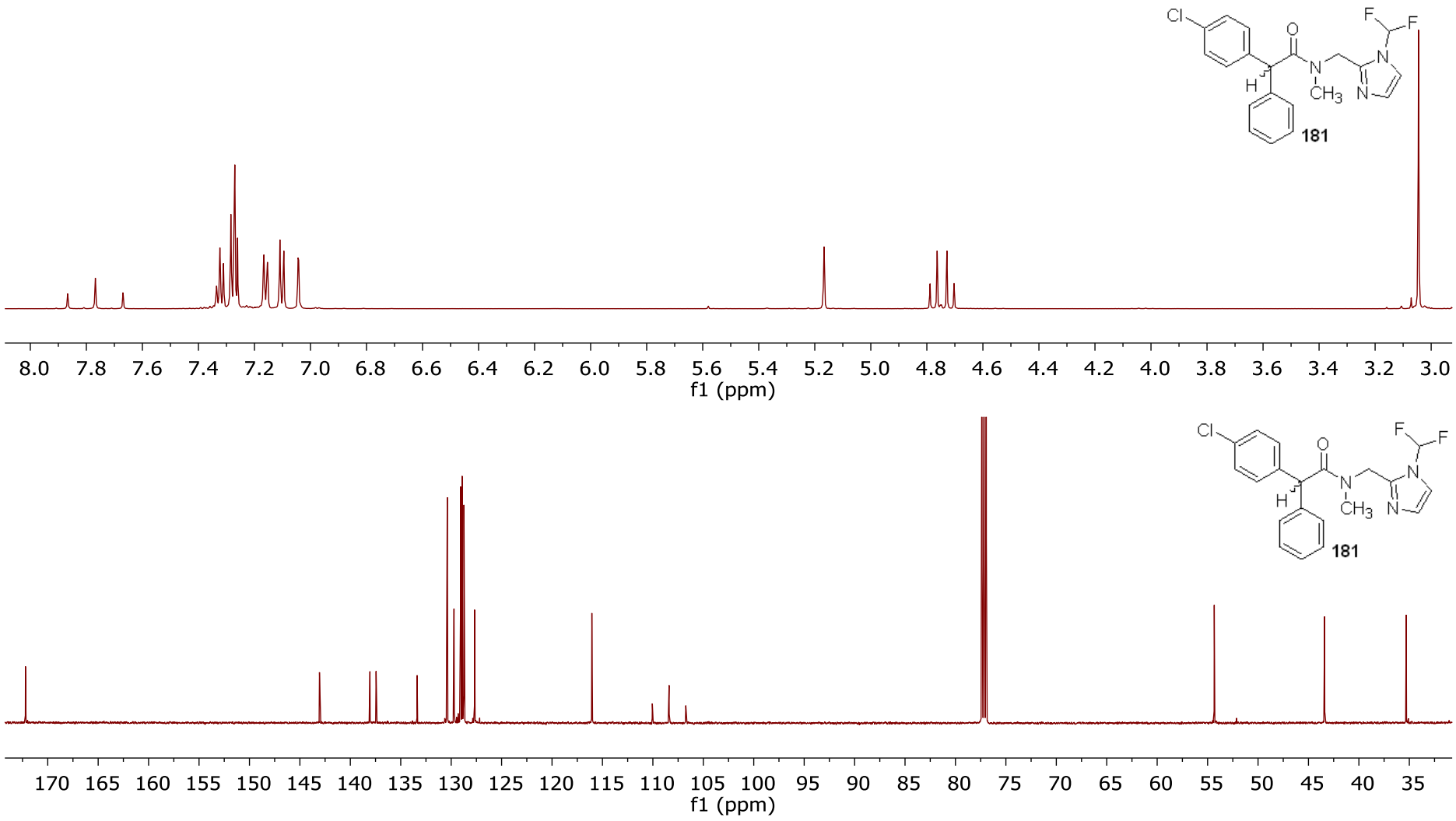

176
f1 (ppm)
176
f1 (ppm)

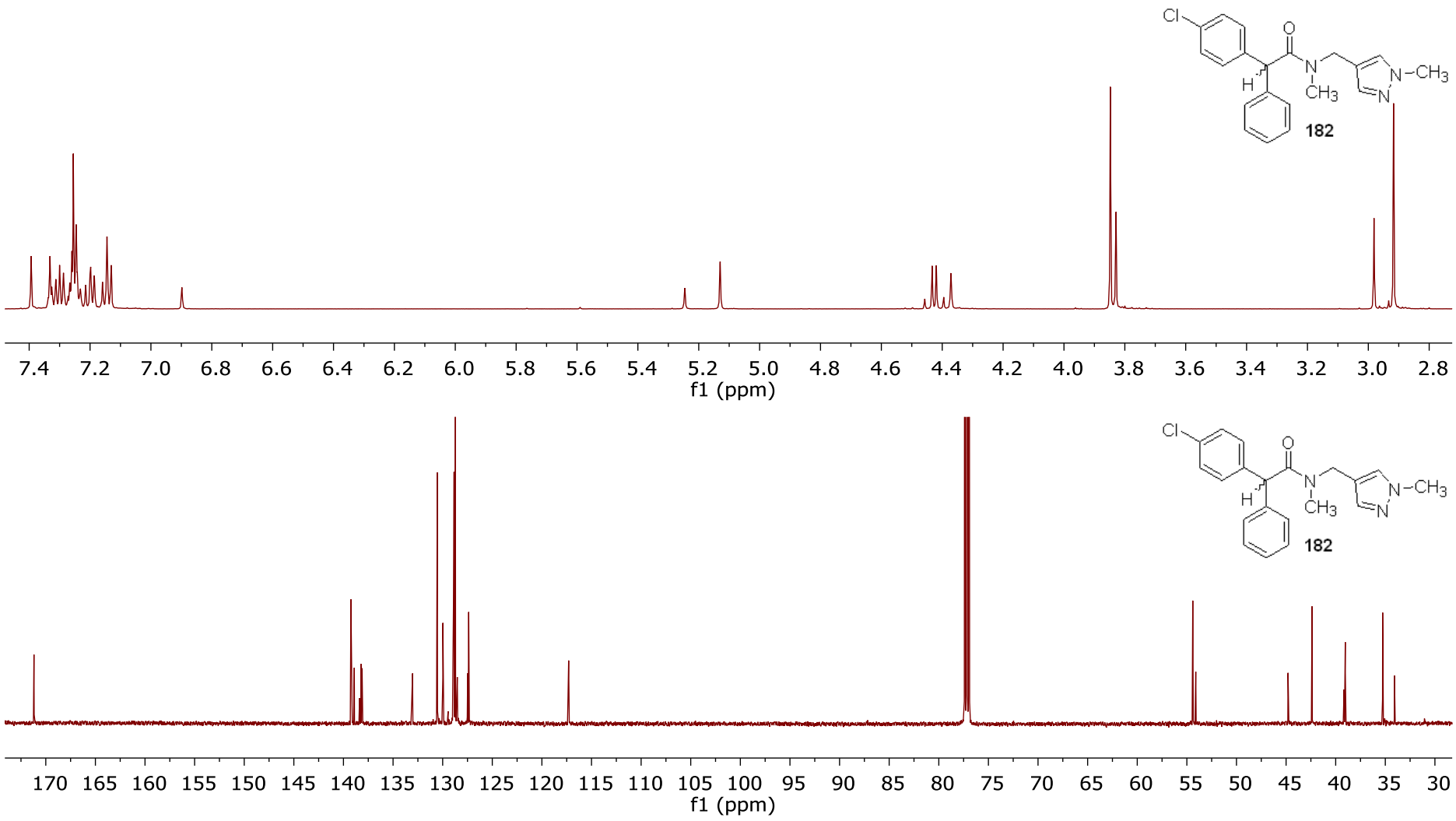

177
f1 (ppm)
177
f1 (ppm)


178
f1 (ppm)
178
f1 (ppm)


OH
N
N
O
H
Cl
179
f1 (ppm)
OH
N
N
O
H
Cl
179
f1 (ppm)


OCH3
N
N
O
H
Cl
180
f1 (ppm)
OCH3
N
N
O
H
Cl
180
f1 (ppm)


Cl
O
F
F
N
H
CH3
N
N
181
8.0 7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0
f1 (ppm)
Cl
O
F
F
N
H
CH3
N
N
181
170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35
f1 (ppm)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N
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CH3
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f1 (ppm)
Cl
O
N
CH3
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CH3
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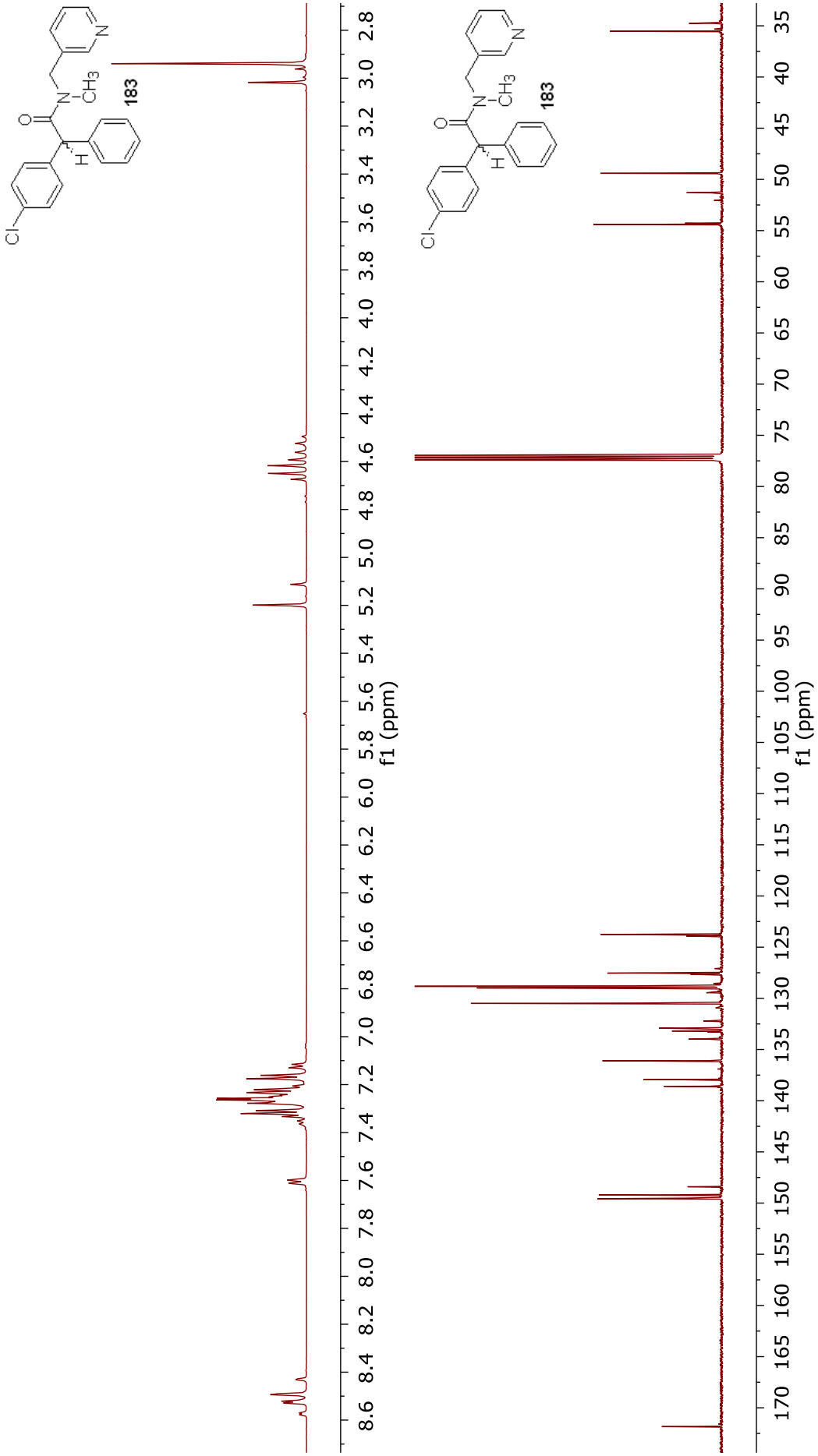
f1 (ppm)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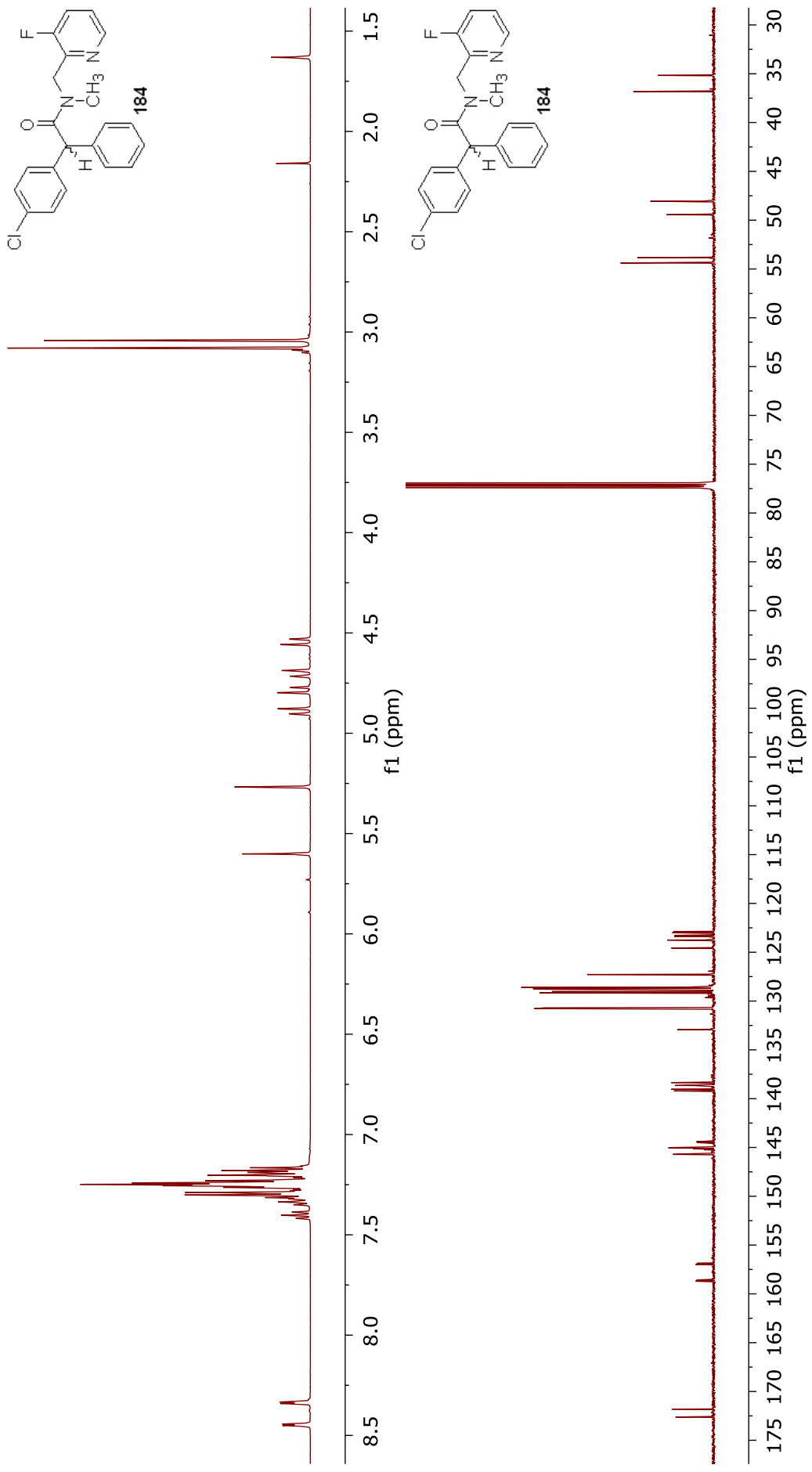
f1 (ppm)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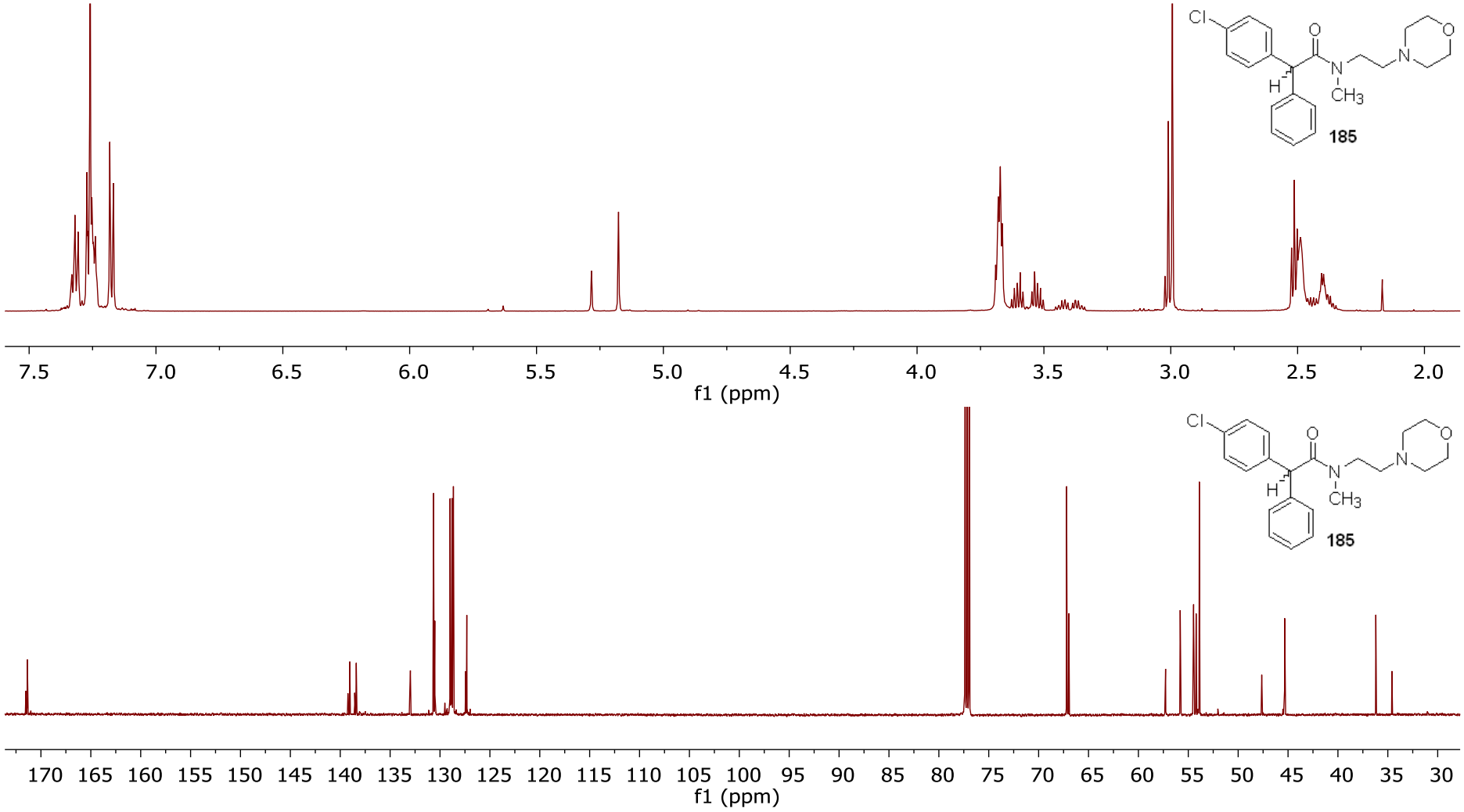
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f1 (ppm)
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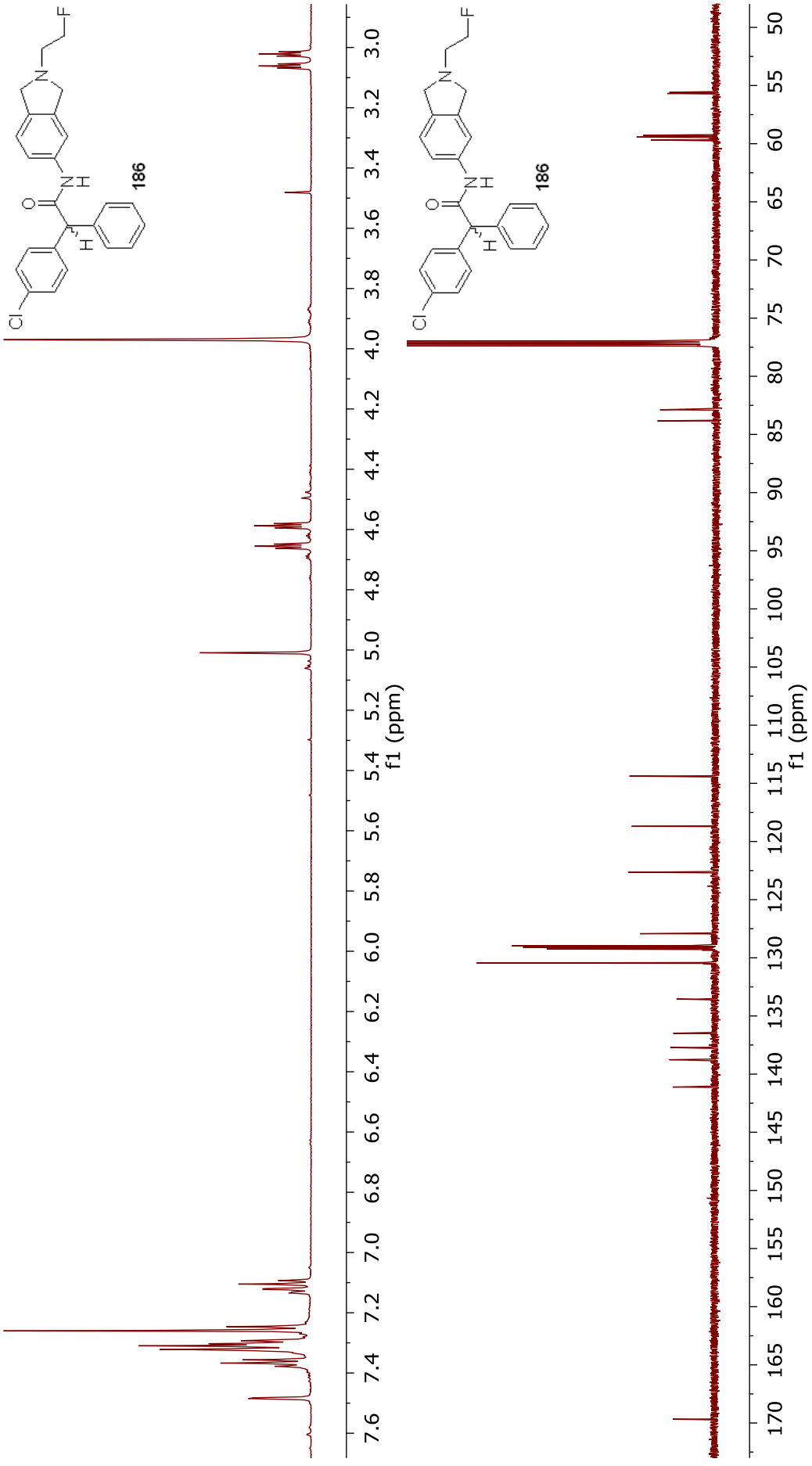
f1 (ppm)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5.0
4.8
4.6
4.4
4.2
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3.8
3.6
3.4
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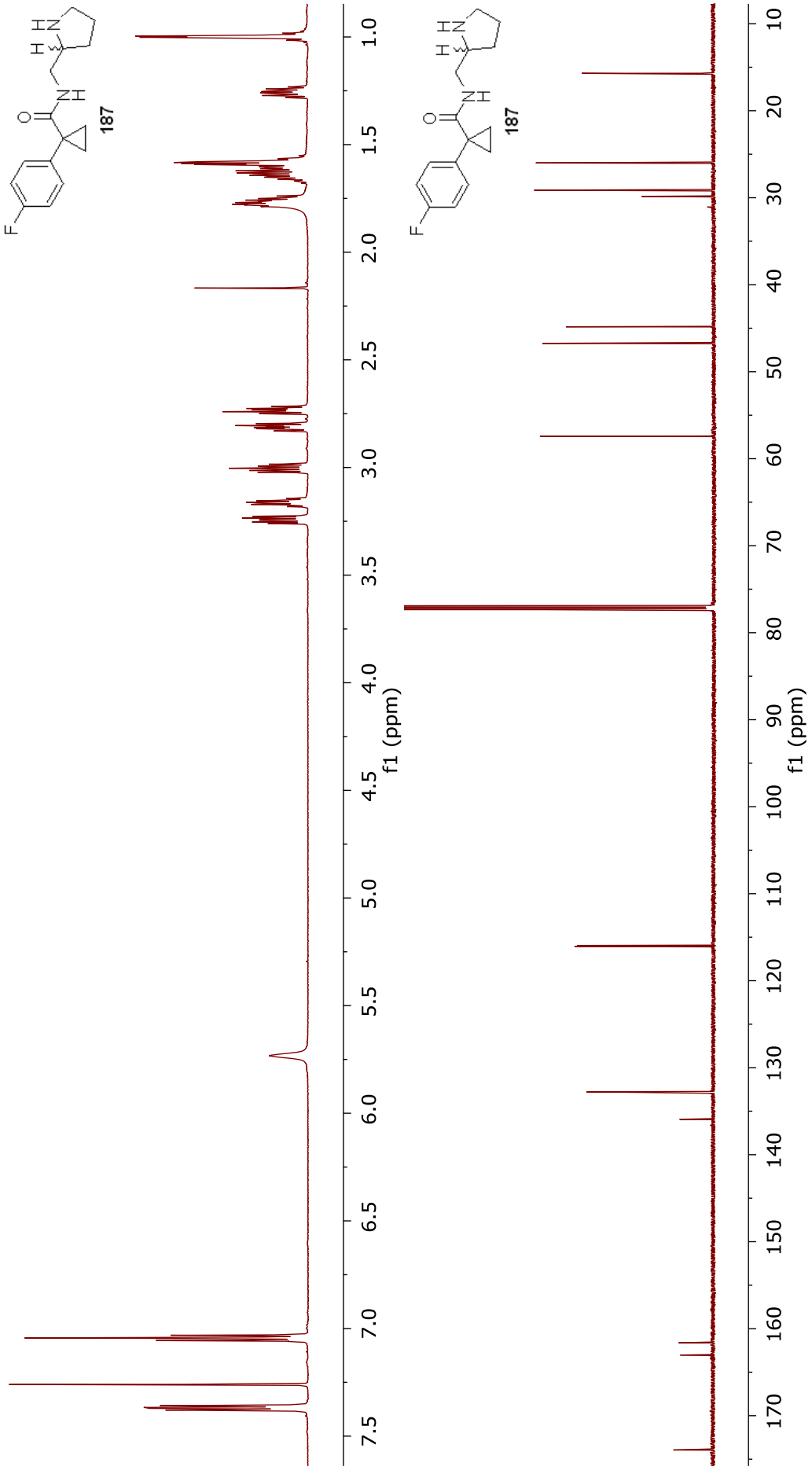
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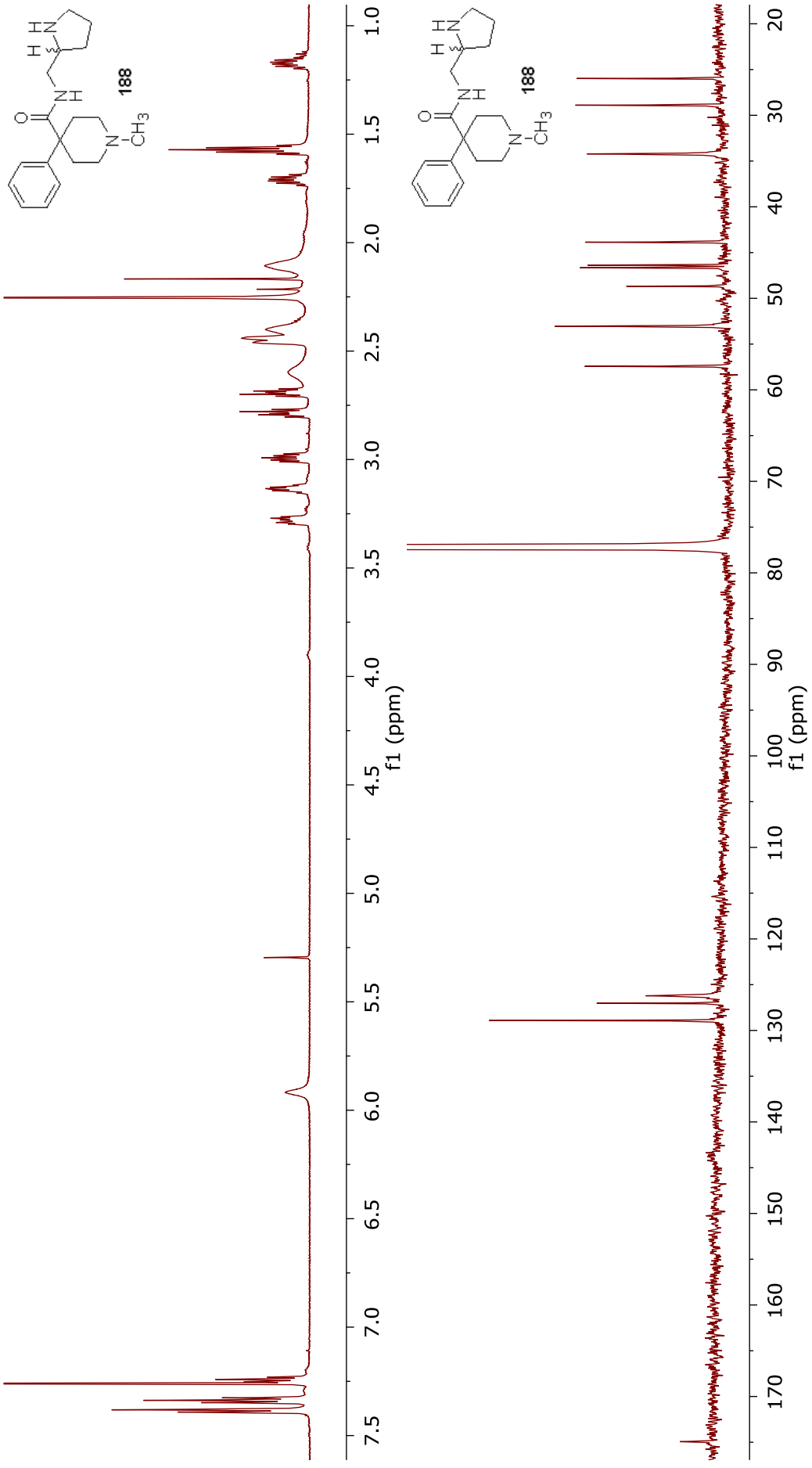
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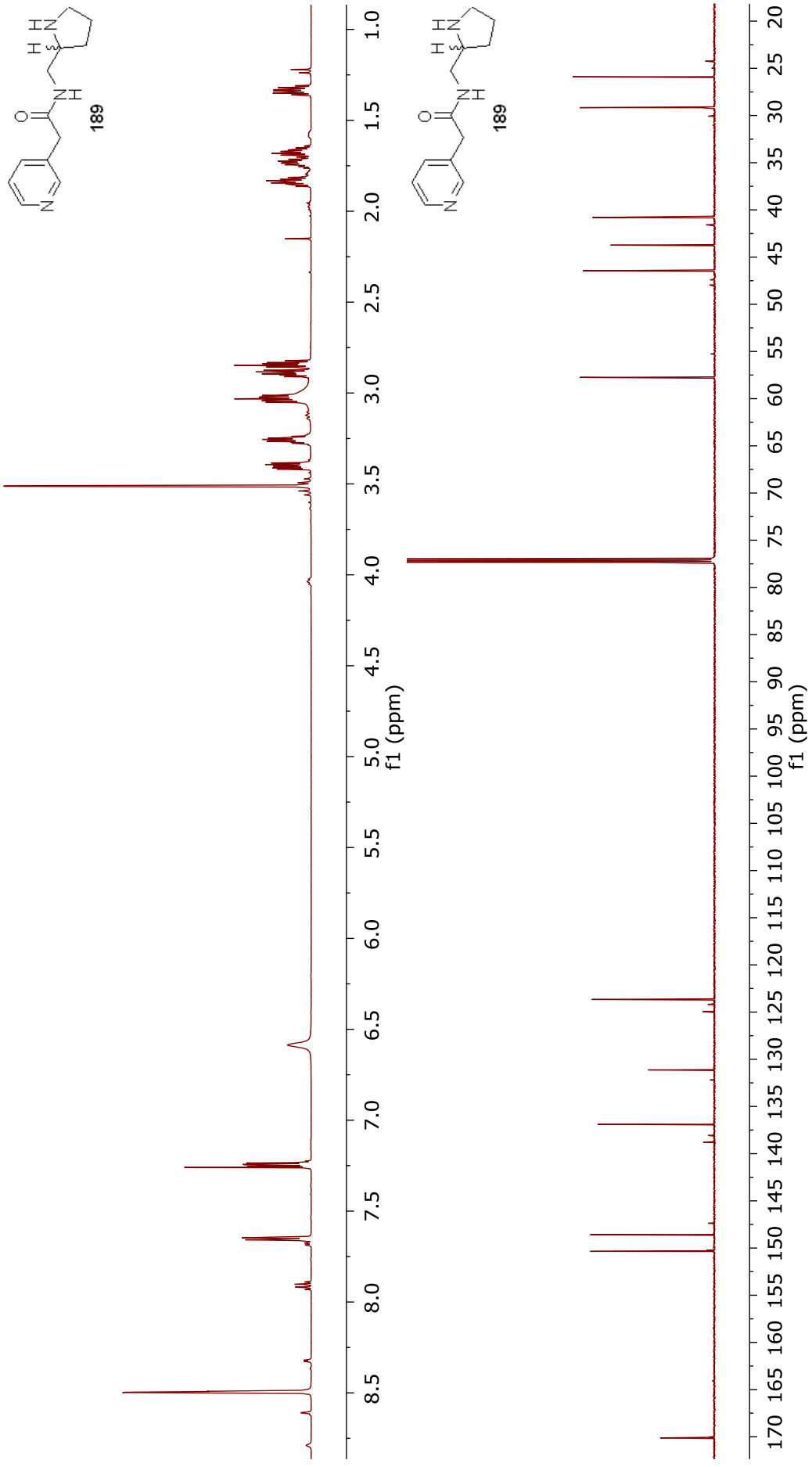
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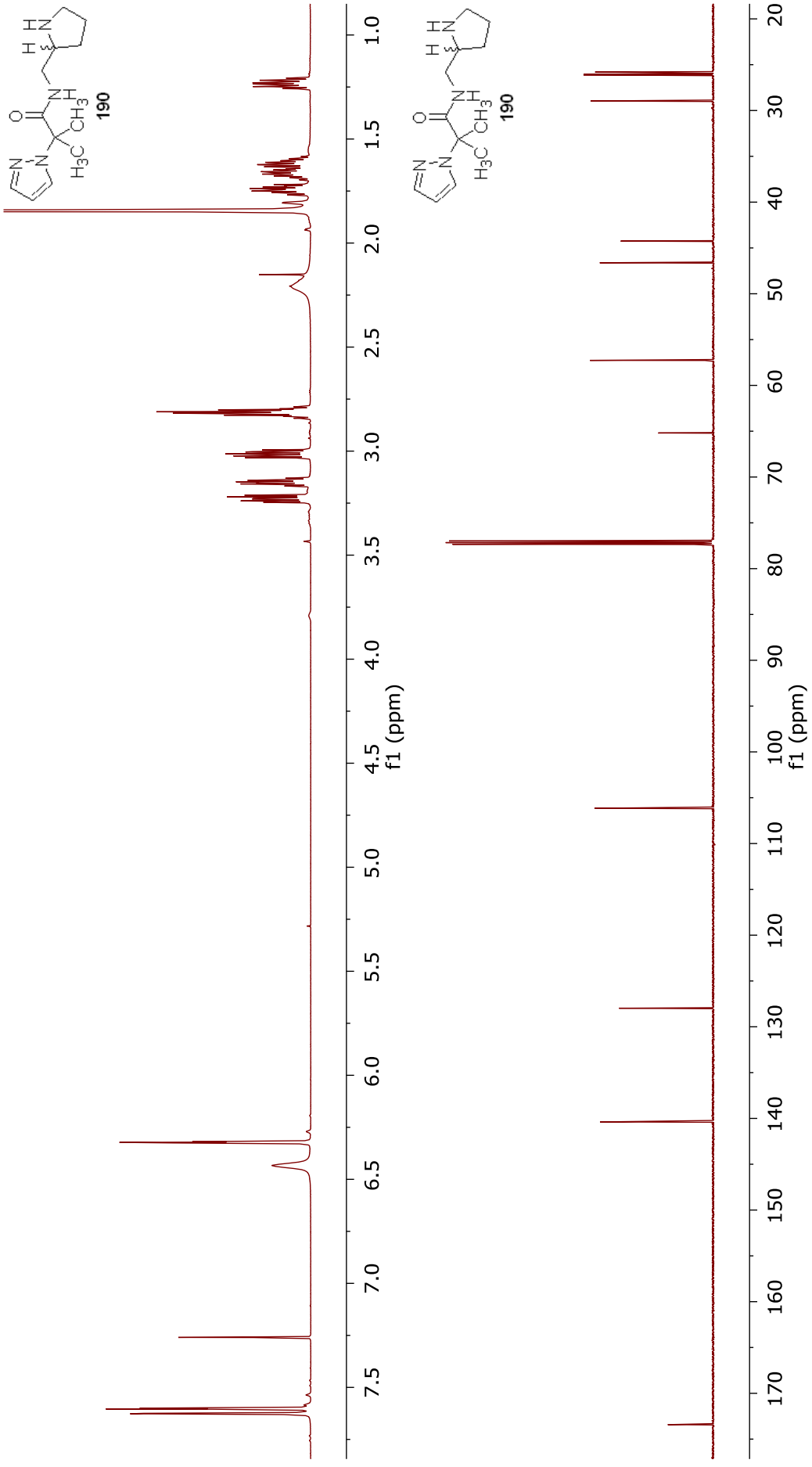
f1 (ppm)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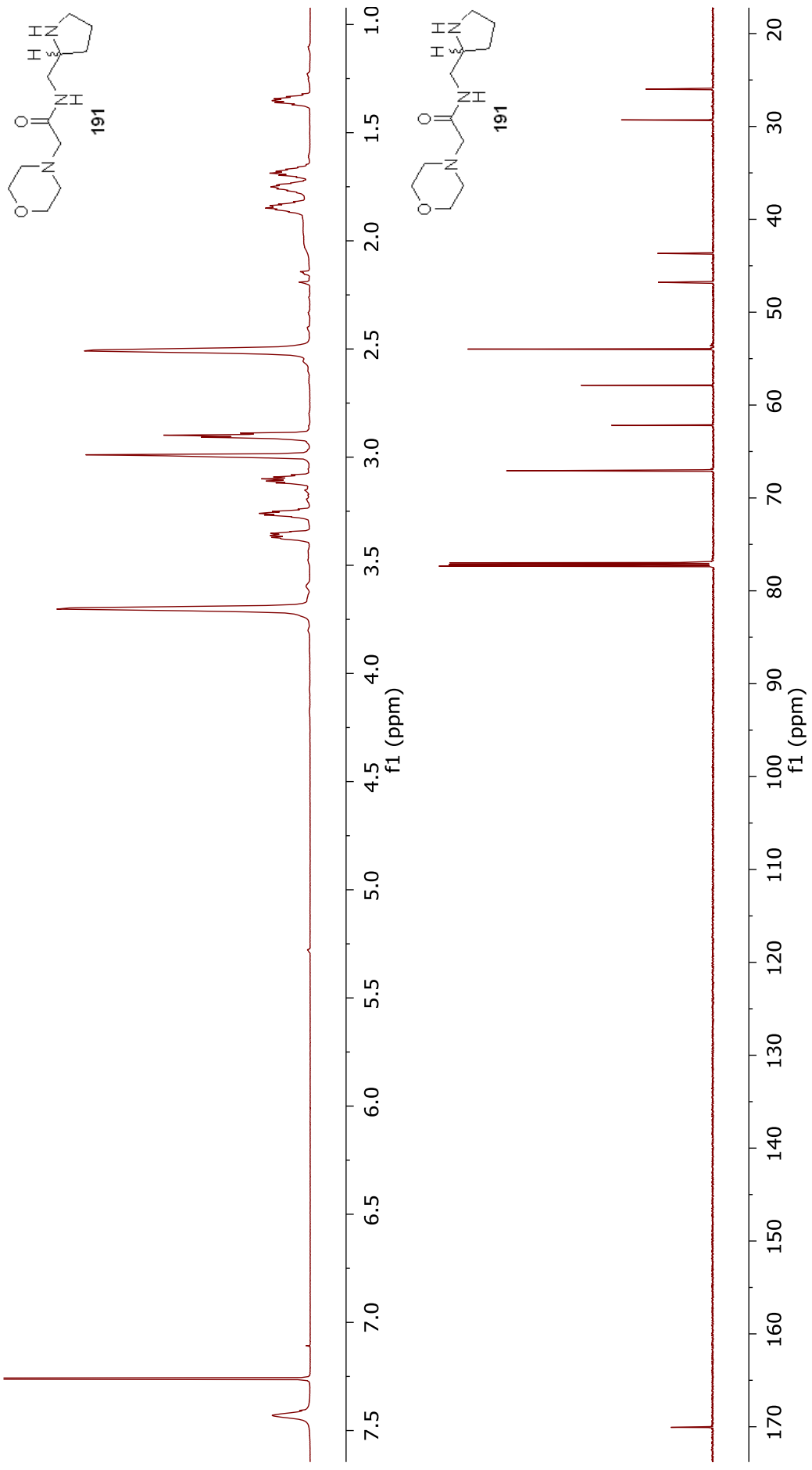
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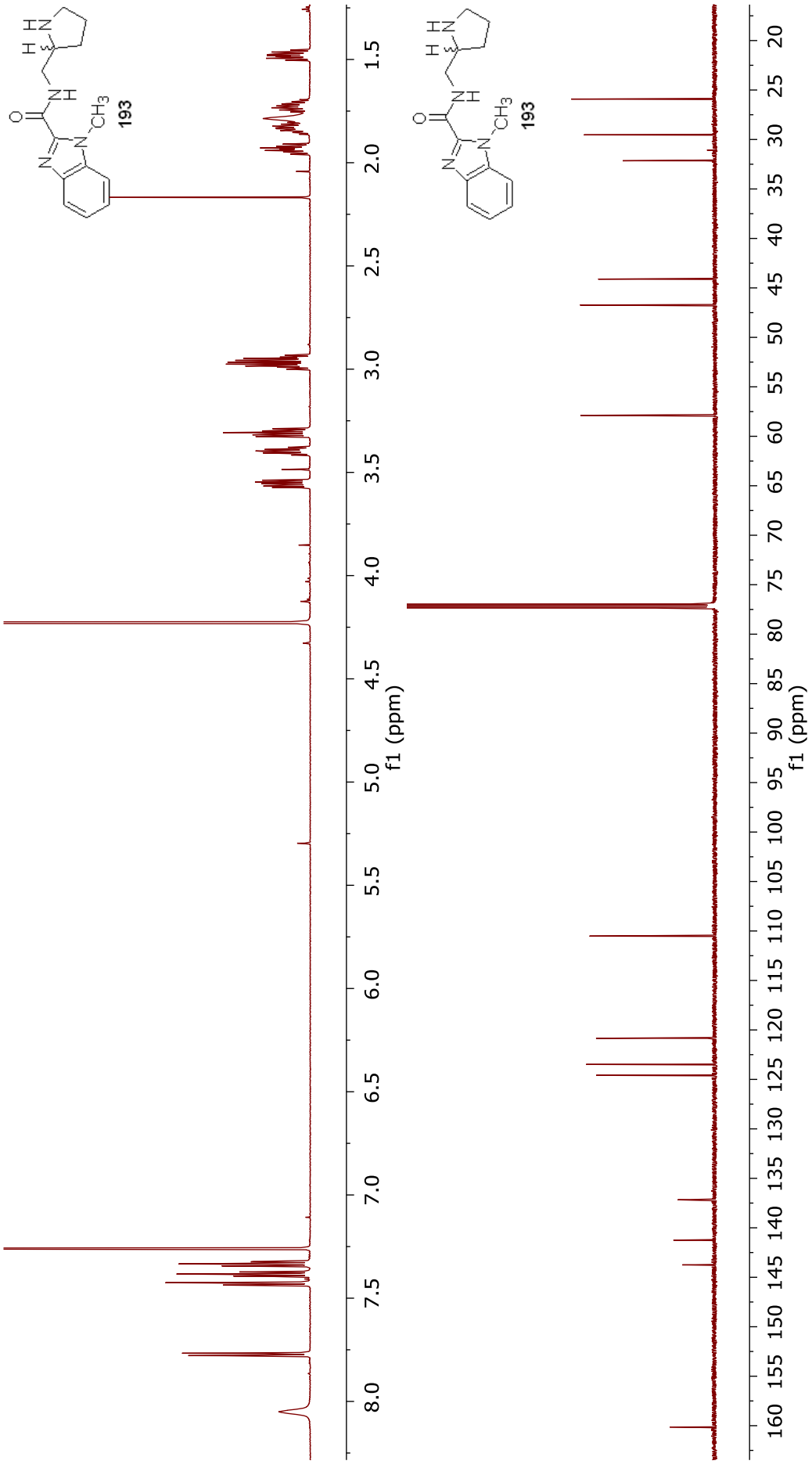
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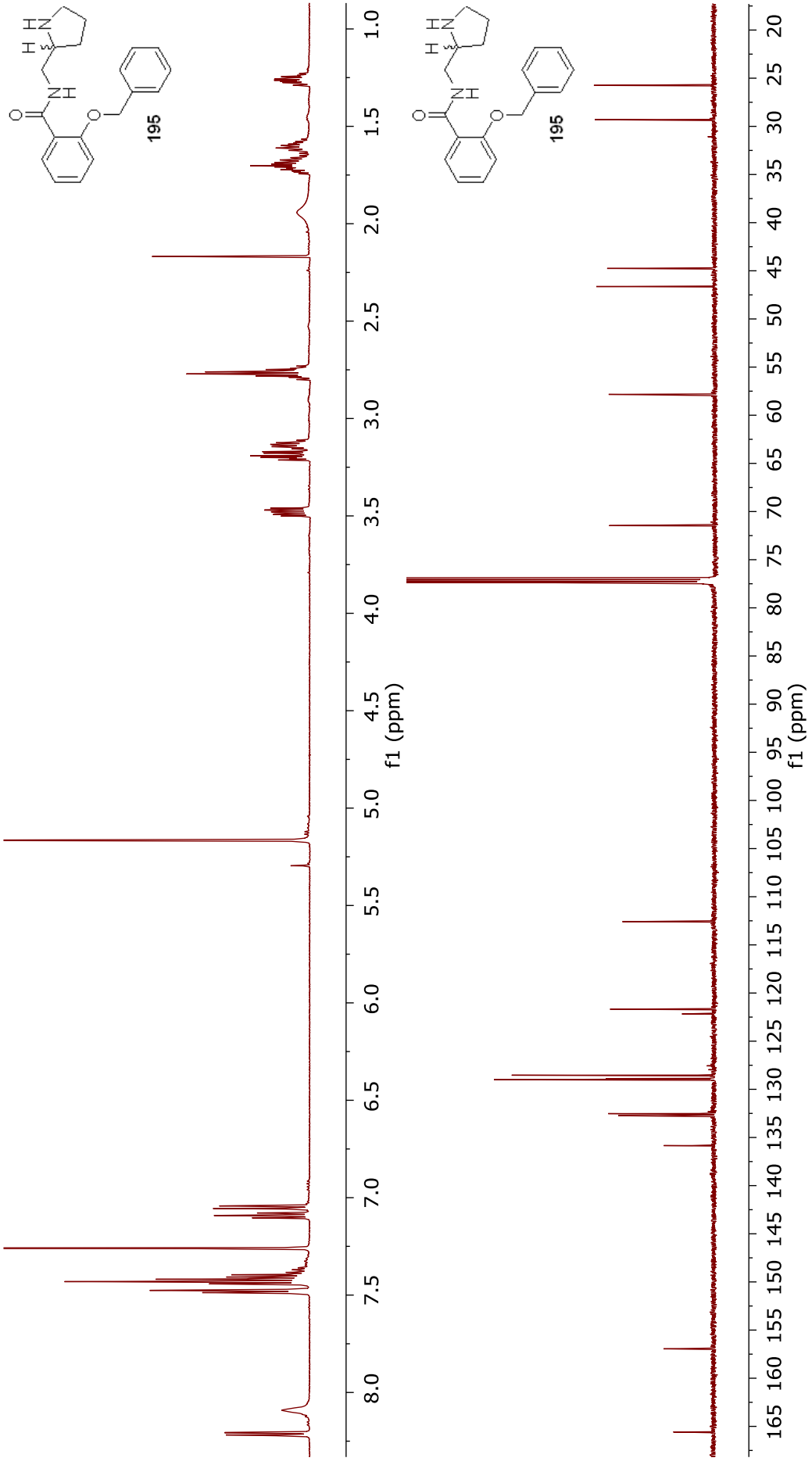
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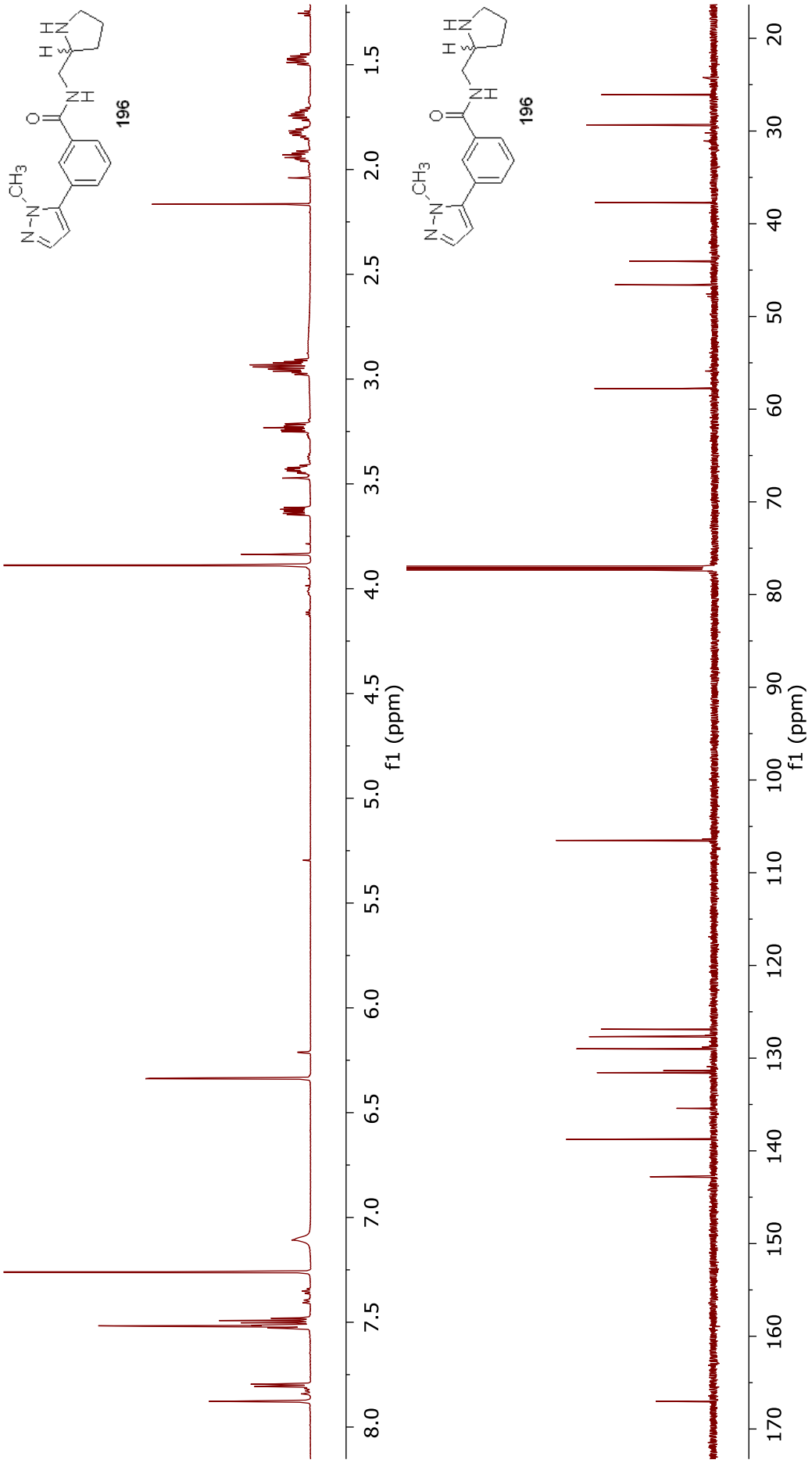
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f1 (ppm)
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f1 (ppm)

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